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Health and Environmental Effects Profile for Dinitrotoluene

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16. ABSTRACT

The Health and Environmental Effects Profile for Dinitrotoluene was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Dinitrotoluene has been evaluated as a carcinogen. The human carcinogen potency factors (q_1^*) for 2,4-, 2,6- and technical dinitrotoluene are 6.8×10^{-1}, 2.3×10^{-1} and 2.3×10^{-1} $(mg/kg/day)^{-1}$, respectively, for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for 2,4-, 2,6- and technical dinitrotoluene is 100. Existing data are insufficient to determine an RQ value for 2,3-, 2,5- and 3,4-dinitrotoluene.

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR DINITROTOLUENE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
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This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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EXECUTIVE SUMMARY

The 2,3-, 2,4-, 2,5-, 2,6- and 3,4- isomers of DNT are reviewed in this document. All five isomers are solid at room temperature (Aldrich, 1984; Verschueren, 1983; Weast, 1973). The water solubility and vapor pressure of one of the more common isomers, 2,4-DNT, is 300 ppm at 22°C (Dunlap, 1981) and 1.1×10^{-4} mm Hg at 20°C (Spanggord et al., 1980a), respectively. From the 1977 TSCA inventory data (U.S. EPA, 1977), it appears that 2,4-DNT has the highest production volume, followed by 2,6-DNT. Assuming that a commercially-produced mixture of 2,4- and 2,6-DNT is 79% 2,4-DNT and 19% 2,6-DNT (Dunlap, 1981), then $\sim 5.69 \times 10^{8}$ and 1.37×10^{8} pounds of 2,4- and 2,6-DNT, respectively, were produced in the United States in 1982 (USITC, 1983). DNTs are used in organic synthesis, dyes, explosives (Hawley, 1981) and as a propellant additive (Sears and Touchette, 1982). A major use of 2,4-DNT is the making of 2,4-diaminotoluene (Hoff, 1983).

DNTs released to water will remain primarily in the water column. The measured log K_{ow} value for 2,4-DNT is 1.98 (U.S. EPA, 1984a). Calculated log K_{ow} values for the other isomers range from 1.72-2.28 (U.S. EPA, 1984a). These intermediate log K_{ow} values suggest that DNTs in water will have a slight tendency to sorb to sediments, suspended solids and biota. This is supported by experimental data by Spanggord et al. (1980b), which indicated that biosorption and sediment sorption would not be significant in the environment. Volatilization of DNTs from water will not be a significant transport mechanism. For example, Spanggord et al. (1980b) estimated the volatilization half-life of 2,4-DNT from a body of water as 438 days.

In water, photolysis and biodegradation will be the primary degradation mechanisms. Photolysis half-lives for 1.0 mg/ℓ 2,4-DNT were 43 hours in distilled water and 2.7, 9.6 and 3.7 hours in river, bay and pond waters, respectively (Spanggord et al., 1980b). Biodegradation rates found in the literature varied widely, probably because of different seed, acclimation schedules (or lack of) and DNT concentrations. The latter point is important because DNT is inhibitory at relatively low concentrations. For example, 2,4-DNT at 10 mg/ℓ was inhibitory to municipal activated sludge organisms (Davis et al., 1981). Spanggord et al. (1980b) found that natural surface water microorganisms could use 10 mg/ℓ 2,4-DNT as a sole carbon source. After a 2- to 3-day lag period, >90% of 10 mg/ℓ 2,4-DNT was transformed in aerated water in 6 days of incubation. This same culture was acclimated to grow on 120 mg/ℓ 2,4-DNT. Hydrolysis will probably not be an important fate process for DNTs. For example, 2,4-DNT concentration did not change after 2 weeks in sterile, natural water (Spanggord et al., 1980b).

Bailey (1982) estimated a half-life of 11.0 hours for 2,4-DNT in natural waters. Zoeteman et al. (1980) estimated the half-life of 2,4-DNT as 1.7 days in the Rhine River. The other DNT isomers will probably behave similarly.

The atmospheric half-life for each of the DNT was estimated as 8.0 hours (U.S. EPA, 1985). In the soil, DNT will be slightly mobile, with K_{oc} values estimated from 205-414 (Lyman et al., 1982). DNTs may biodegrade in soils based on comparison with aquatic biodegradation studies.

DNTs are commonly found in wastewater from TNT production. For example, 2,4-DNT and 2,6-DNT average concentrations in wastewater from TNT production were 9.7 and <0.3 mg/ℓ, respectively (Spanggord and Suta, 1982). DNT was detected in some samples from the Delaware River basin at >1 μg/ℓ

(DeWalle and Chian, 1978). 2,4-DNT concentrations in Dokai Bay (Japan) ranged from undetectable to 206 µg/ℓ (Hashimoto et al., 1982). Because of insufficient data on DNT concentrations in drinking water and food, average daily intakes could not be calculated. DNT air concentrations in the workplace ranged from undetectable to 0.042 mg/m³ at the Du Pont Chambers worksite in Deepwater, NJ (Fannick, 1981) and at the Olin Chemical Company in Brandenburg, KY, from undetectable to 0.42 mg/m³ (Ahrenholz, 1980). Information regarding dermal exposure from DNTs could not be located in the available literature as cited in the Appendix.

The DNTs appear to be rapidly and extensively absorbed through the skin, respiratory tract and gastrointestinal tract in most species, although the available literature predominantly concerns oral absorption. Up to 90% of an oral dose of DNT was absorbed in rats within 24 hours (Hodgson et al., 1977) and maximum absorption occurred in the small intestine ~6 hours after administration (Mori et al., 1978). The observed extent of oral absorption for DNT isomers was 2,4- ≈ 3,4- > 3,5- ≈ 2,5- > 2,3- ≈ 2,6-DNT (Hodgson et al., 1977). Mice were an exception since only 8-12% of an oral dose of 2,4-DNT was taken up by the intestine (TSCA-ITC, 1981).

Distribution studies have established that there is some accumulation of radioactivity from radiolabeled DNT isomers in the liver, kidneys, skin and adipose tissue (Mori et al., 1977; Hodgson et al., 1977; Ellis et al., 1979; Lee et al., 1978) in most species. Peak hepatic concentrations of radioactivity occurred 1-2 hours and 8-12 hours after oral administration of ^{14}C-2,4- or ^{14}C-2,6-DNT with radioactivity from the 2,6-isomer retained to a greater extent (Rickert et al., 1983). A similar pattern of distribution was also observed in rabbits, dogs and monkeys (Lee et al., 1978).

Metabolism of DNT isomers is extensive and occurs almost exclusively in the gut and liver of all species studied. In vitro studies have established that, under physiological conditions, oxidative metabolism predominates in the liver leading to the formation of dinitrobenzyl alcohol followed by conjugation to the corresponding glucuronide (Decad et al., 1982; Dent et al., 1982; Bond and Rickert, 1981; Bond et al., 1981; Long and Rickert, 1982). This glucuronide represents the major fraction of DNT metabolites excreted into the bile; males generally excreted more by this route than females (Medinsky and Dent, 1983).

Intestinal microflora from a number of species, including humans, are effective in reducing DNTs to monoaminonitrotoluenes and diaminonitrotoluenes (Dent et al., 1982; Guest et al., 1982; Mori et al., 1985; Guest et al., 1982; Mori et al., 1984a). Reductive DNT metabolism by gut microorganisms is estimated to occur at a rate ~1000 times greater than that observed in isolated hepatocytes (Guest et al., 1982).

Urinary DNT metabolites consist chiefly of N-acetylaminonitrobenzoic acid, dinitrobenzoic acid, aminonitrobenzoic acid and dinitrobenzyl alcohol glucuronide derivatives (Long and Rickert, 1982; Medinsky and Dent, 1983; Rickert and Long, 1981), which indicates that both reductive and oxidative metabolism occur in vivo (Mori et al., 1981). A major sex difference exists in the excretion of the glucuronide, the most common urinary metabolite; up to twice as much of this metabolite is found in urine of females (Medinsky and Dent, 1983; Rickert and Long, 1981). Axenic rats were used to demonstrate the importance of gut microflora to the formation of metabolites undergoing oxidation and reduction (Rickert et al., 1981), while other studies reported that intestinal metabolism is related to the extent of DNT-derived compounds covalently binding to liver macromolecules (Rickert et al., 1981, 1983; Long and Rickert, 1982). Covalent binding of DNT-derived compounds to hepatic macromolecules is consistently more prevalent in males and may be associated with the greater concentrations of glucuronide metabolites transported in the bile of males compared with females (Long and Rickert, 1982; Medinsky and Dent, 1983).

A hypothesis presented by Bond et al. (1981) explains the general metabolic scheme for the DNT isomers. The compound initially is metabolized to a nitrobenzyl alcohol glucuronide in the liver and transported preferentially in the bile (males) or in the general circulation and urine (females). Once in the intestine, the glucuronide moiety is hydrolyzed and the resulting alcohol is reduced to a monoaminonitrotoluene, which is reabsorbed and returned to the liver. Covalent binding to DNA, RNA or protein or further transformation to a reactive electrophile then occurs within the liver. This enterohepatic scheme reflects results obtained in both in vivo and in vitro studies and can reconcile the sex differences observed in DNT metabolism.

Preliminary data indicate that human DNT metabolism is similar to that observed in rats, at least for 2,4-DNT, (Rickert et al., 1984).

Excretion of DNT isomers occurs almost exclusively through urine and feces although the relative contribution of each route is species and strain dependent. In most species, urinary excretion predominates with 50-80% of the dose eliminated in the urine within 24 hours, while the feces contained 10-30% of the administered dose after 72 hours (Rickert et al., 1984; Long and Rickert, 1982; Rickert and Long, 1981).

Several bioassays have been performed regarding the carcinogenicity of DNT and individual DNT isomers. Rats exposed to 80 and 200 ppm 2,4-DNT in the diet for 78 weeks were reported to have a significant dose-related increased incidence of subcutaneous fibromas in males and statistically significant increased frequency of mammary gland fibroadenomas in high-dose females compared with controls. Male and female mice exposed to 80 and 400 ppm in the diet for 78 weeks displayed no evidence of a significant tumor incidence in any tissue relative to control groups (NCI, 1978).

Administration of 2,4-DNT to rats at dietary concentrations of 0, 15, 100 and 700 ppm for 2 years resulted in an increase in the number of high-dose animals with hepatocellular carcinomas that was statistically significant only in females (Ellis et al., 1979). High-dose females also had a 97% incidence of benign mammary gland neoplasms. This study also reported a statistically significant increase in the number of high-dose male mice with benign (cystic papillary adenomas) and malignant (solid renal cell carcinomas, cystic papillary carcinomas) renal tumors following dietary administration of 0, 100 or 700 ppm 2,4-DNT. Hepatomas were also observed in these mice but the incidence was not dose-related. Female mice maintained on the same diet had no treatment-related increase in tumor frequency in any tissue.

A 100% incidence of hepatocellular carcinoma was reported in male rats exposed to 35 mg/kg/day technical grade DNT (76% 2,4-DNT, 19% 2,6-DNT) in the diet after 1 year of treatment, while females had a 50% incidence (CIIT, 1982). Rats treated with 0, 3.5 and 14 mg/kg/day technical grade DNT in the diet and sacrificed after 78 and 104 weeks showed dose-dependent increases in these and other lesions including hepatic neoplastic nodules, testicular mesotheliomas, mammary gland fibroadenomas and adenomas, and subcutaneous fibromas.

Recent investigations utilizing initiation/promotion protocols in rat liver and mouse skin have strongly indicated that 2,6-DNT is the isomer most responsible for the carcinogenic response observed in previously mentioned studies (Popp and Leonard, 1982; Leonard et al., 1983; Slaga et al., 1985).

These studies have further suggested that both 2,4- and 2,6-DNT are able to enhance the formation of neoplasms. Popp and Leonard (1982) briefly reported that dietary intake of 7 and 14 2,6-DNT/kg/day resulted in a 100 and 95%, respectively, incidence of hepatocellular carcinomas, while a dose of 27 mg 2,4-DNT/kg/day was ineffective at producing tumors. There is therefore a strong possibility that 2,6-DNT is the primary carcinogen in technical grade DNT and it may also contribute to the effects observed in the studies using 2,4-DNT as a contaminant representing 2-5% of the administered dose.

There was no indication of any teratogenic potential for technical grade DNT in rats (Price et al., 1985) or 2,4-DNT in rats (Ellis et al., 1979) or mice (Smith, 1983) at doses that did not produce significant maternal toxicity. In the most comprehensive teratologic study (Price et al., 1985), evidence of embryo/fetal toxicity was observed only at a dose (150 mg/kg/day technical grade DNT) that also produced 46.2% maternal mortality.

A significant reduction was reported in the sperm counts of workers exposed to DNT compared with nonexposed workers, although the control values were unusually high (Ahrenholz, 1980). A similar study involving more workers at a different plant found no differences between exposed and control groups in fertility, testicular volume, sperm count and sperm morphology (Hamill et al., 1982). Testicular degeneration and decreased spermatogenesis have been reported to occur following subchronic and chronic exposure to 2,4-DNT in rats (Lee et al., 1985) mice (Hong et al., 1985) and dogs (Ellis et al., 1985). Similar effects were observed in 13-week studies using 2,6-DNT in mice, rats and dogs (Lee et al., 1976).

Treatment of male and female mice with up to 34 and 45 mg/kg/day of 2,4-DNT, respectively, for 6 months did not result in any significant changes in fertility or reproductive performance in rats (Ellis et al., 1979).

An assessment of the data generated by genetic and reproductive toxicity studies suggests that while 2,4- and 2,6-DNT can produce testicular damage, they do not appear to be genetically active in male germ cells. The data are currently insufficient to assess the reproductive hazard of the DNTs in females, but there is limited evidence that suggests ovarian toxicity.

Chronic and subchronic toxicity studies have revealed that the blood, liver, testis and neuromuscular system are the primary organs affected by the DNTs. Decreases in average body weight gain were the only significant effects observed in mice and rats exposed to relatively low levels of 2,4-DNT (0.04% and 0.02%, respectively) in the diet in a 2-year NCI (1978) bioassay. The major adverse effect in dogs treated with 2,4-DNT for 13 weeks or 2 years was a neuropathy characterized by incoordination and paralysis. Central nervous system lesions were also seen in some affected dogs given 1.5 mg/kg/day for 2 years, 10 mg/kg/day for 6 months and 25 mg/kg/day for 2 months. Less important adverse effects included methemoglobinemia and its sequelae, testicular atrophy and biliary tract hyperplasia (Ellis et al., 1985).

In mice, 2,4-DNT is apparently more toxic to males than females. Feeding 2,4-DNT in the diet for 13 weeks at concentrations of 0.07 and 0.2% resulted in decreased weight gain in males but not females (Hong et al., 1985). A high dose of 0.7% resulted in depressed body weight, mild anemia and mild hepatocellular dysplasia in both sexes as well as moderate testicular atrophy in males. Chronic exposure of mice to dietary concentrations of 0.01, 0.07 or 0.7% 2,4-DNT resulted in a high incidence of epithelial renal tumors and hepatocellular dysplasia in all treated males, testicular atrophy in middle- and high-dose males and toxic anemia and increased mortality in the high-dose male group. Serious adverse effects in females occurred primarily in the high-dose group and consisted of toxic anemia, hepatocellular dysplasia, nonfunctioning follicles with a lack of corpora lutea, and death. In addition, a generalized pigmentation, most likely related to 2,4-DNT, was observed in several tissues in all treated mice, especially in the high-dose groups.

Treatment of rats with 2,4-DNT in the diet at concentrations of 0.07, 0.2 or 0.7% for 13 weeks caused a depression in weight gain in all low-dose animals and weight loss in middle- and high-dose rats (Lee et al., 1985). Relative changes in organ weights, decreased spermatogenesis, reticulocytosis and splenic hemosiderosis, and demyelination in the cerebellum and brainstem were observed in middle- and high-dose rats. In addition, high-dose males and females were anemic and had increased mortality. Chronic treatment of rats for 2 years suggested that a dietary concentration of 0.0015% produced no apparent toxicity. A dose of 0.01% in the diet, however, was moderately toxic while a concentration of 0.07% was quite toxic and shortened life span. These dose levels were associated with a progressive development of hepatic hyperplastic foci to hepatocellular carcinomas, atrophy of seminiferous tubules and decreased spermatogenesis, an increased incidence of subcutaneous tumors, toxic anemia with reticulocytosis and indications of mild neuromuscular effects (Lee et al., 1985). Feeding rats a diet containing a relatively high concentration of 2,4-DNT (0.5%) for 6 months resulted in a significant weight loss, a high rate of mortality, hepatic hypertrophy, testicular atrophy and pronounced methemoglobinemia (Kozuka et al., 1979).

A study by CIIT (1982) reported the effects of feeding technical grade DNT to rats for 104 weeks at dose levels of 3.5, 14 or 35 mg/kg/day.

General signs of toxicity including decreased weight gain, alterations in the relative weights of several organs and various histologic tissue alterations occurred in all dose groups of both sexes. In the middle- and high-dose groups, degenerative and proliferative alterations of the hepatocytes and bile duct epithelium were observed within the first 26 weeks of treatment. These alterations generally involved necrotic or vacuolated hepatocytes and necrosis and hyperplasia of the biliary epithelium. These lesions were generally more prevalent in males, were dose-related and progressed throughout the study. A high incidence of hepatotoxicity, and hepatic neoplasms, mammary fibroadenomas and subcutaneous fibromas, neurofibromas and sarcomas were also observed. In addition, a low grade regenerative anemia occurred in the high-dose groups.

The subchronic toxicity of 2,6-DNT has been discussed only in an abstract with very few details provided (Ellis et al., 1976). The effects in dogs, mice and rats were reportedly similar to those seen in short-term studies using 2,4-DNT (Hong et al., 1985; Lee et al., 1985; Ellis et al., 1985). Doses of 4, 20 or 100 mg/kg/day for dogs and 0.01, 0.05 or 0.25% in the diet for rats and mice exerted effects primarily on the red cells (toxic anemia), nervous system (incoordination, demyelination) and testes (atrophy) and decreased spermatogenesis). Other affects included weight loss or decreased weight gain and increased mortality.

The acute toxic effects of DNT most commonly include central nervous system depression, respiratory depression and ataxia. Oral LD_{50} values range from 177 mg/kg for 2,6-DNT in rats (Vernot et al., 1977) to 1954 mg/kg for 2,4-DNT in male mice (Ellis et al., 1980). Generally, the data suggest that mice and rats were more susceptible to the acute effects of 3,5-DNT compared with the other isomers. Mice appeared somewhat more resistant to the effects of all DNT isomers compared with rats (Ellis et al., 1980).

Cats are especially sensitive, having an oral LD_{Lo} of 27 mg/kg for 2,4-DNT (Spector, 1956). Eye and skin irritation effects for all isomers are, at most, minimal (Ellis et al., 1980).

In humans, a number of signs and symptoms including headache, vertigo, fatigue, dizziness, weakness, nausea, vomiting, dyspnea, drowsiness, arthralgia, insomnia, tremor, paralysis, unconsciousness, chest pain, shortness of breath, palpitation, anorexia, weight loss and an unpleasant metallic taste in the mouth reportedly resulted from 2,4-DNT exposure (Floret, 1929; Lewin, 1921; McGee et al., 1942; Perkins, 1919; TSCA-ITC, 1981; U.S. EPA, 1980a). Medical examinations of 2,4-DNT-exposed workers indicated impaired reflexes, nystagmus, tremors, pallor, cyanosis, anemia. leukocytosis, hypertension, dermatitis, leukopenia and hepatitis or jaundice (Floret, 1929; McGee et al., 1942). Improved plant hygiene and worker awareness apparently lessened or eliminated many of these effects (McGee et al., 1947).

The 2,3-, 2,5- and 3,4-isomers of DNT are apparently more toxic to fish (fathead minnows) and cladocerans (Daphnia magna) than the 2,4-, 2,5- and 3,5-isomers (Liu et al., 1983b). The 2,4-isomer is more toxic to protozoans than the 2,3- or 2,6-isomers (Bringmann and Kuehn, 1981). Algae and protozoa appear to be more sensitive to 2,4-DNT toxicity than fish and invertebrates, with acute toxicity occurring at levels of 0.13-2.7 mg/ℓ, compared with 22-47.5 mg/ℓ for fathead minnows and Daphnia magna.

Among fish species, bluegills and rainbow trout are more sensitive to 2,4-DNT than channel catfish and fathead minnows (Hartley, 1981). Based on the available data, U.S. EPA (1980a) concluded that acute and chronic toxicity to freshwater biota occurred at DNT concentrations as low as 0.330 and 0.230 mg/ℓ, respectively, and that acute toxicity to saltwater organisms occurred at concentrations as low as 0.590 mg/ℓ. More recent data indicating lower adverse effect concentrations than these could not be located in the available literature. The limited information on DNT residues indicates that DNT isomers are not strongly bioaccumulated by aquatic organisms (Bailey and Spanggord, 1983; Hartley, 1981; Liu et al., 1983b), and therefore biomagnification and human exposure from contaminated fish should not be a major concern.

Lee et al. (1978) reported cancer bioassay of 2,4-DNT in male and female CD rats and mice fed 2,4-DNT for 2 years. The major pathologic findings in rats included a significant increase of hepatocellular carcinomas and neoplastic nodules in the liver, and mammary gland tumors of the females. Based on this study, U.S. EPA (1981) estimated a q_1^* for humans of 0.31 $(mg/kg/day)^{-1}$ for 2,4-DNT. This value is extremely close to the value based on Ellis et al. (1979). The slightly higher potency value for Ellis et al. (1979) of 0.68 $(mg/kg/day)^{-1}$ for female SD rats is suggested to conservatively represent the potency of 2,4-DNT.

A human q_1^* of 6.8×10^{-1} $(mg/kg/day)^{-1}$ was derived for 2,4-DNT based on the combined incidence of mammary and liver tumors in female rats exposed to 2,4-DNT in the diet (Ellis et al., 1979). The concentration of 2,4-DNT in water associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are 5.1×10^{-4} and 5.1×10^{-6} mg/ℓ, respectively. A human q_1^* of 2.3×10^{-1} $(mg/kg/day)^{-1}$ was derived from the incidence of hepatic tumors in male rats exposed to technical DNT in the diet (CIIT, 1982). Because the data by Popp and Leonard (1982) indicated that the 2,6-DNT in technical DNT used in the CIIT (1982) study was the primary carcinogenic agent, the q_1^* of 2.3×10^{-1} $(mg/kg/day)^{-1}$ is recommended for estimating the increased lifetime cancer risk of exposure to 2,6-DNT until a definitive bioassay for

2,6-DNT, per se, becomes available. The water concentrations associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are $1.5\text{x}10^{-3}$, $1.5\text{x}10^{-4}$ and $1.5\text{x}10^{-5}$ mg/ℓ, respectively. 2,4-DNT and 2,6-DNT are placed in CAG Group B2 and C, respectively (U.S. EPA, 1984). F factors of 3.98 and 2.83 $(\text{mg/kg/day})^{-1}$ were derived for 2,4- and 2,6-DNT, respectively. Because the F factors fall between 1 and 10, these chemicals are placed in Potency Group 3. A Potency Group 3 chemical with a CAG classification of B2 or C has a MEDIUM ranking for 2,4-DNT and LOW ranking for 2,6-DNT under CERCLA. Lack of data base in support of carcinogenicity for other isomers of DNT places them in CAG Group D.

RQs of 100 based on chronic toxicity were derived for 2,4-DNT, 2,6-DNT and technical DNT. The RQ for 2,4-DNT was based on neurotoxicity in dogs in the chronic study by Ellis et al. (1985). The RQ for 2,6-DNT was derived by analogy to 2,4-DNT. The RQ for technical DNT was based on the reduced survival of rats in the chronic study by CIIT (1982). Data were insufficient to derive RQs for the other DNT isomers.

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
BCF	Bioconcentration factor
BOD	Biological oxygen demand
BUN	Blood urea nitrogen
CAS	Chemical Abstracts Service
CS	Composite score
DNA	Deoxyribonucleic acid
DNT	Dinitrotoluene
EC_{50}	Concentration effective to 50% of recipients
GGT+	Gamma-glutamyltranspeptidase positive
K_{oc}	Soil sorption coefficient
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LD_{LO}	Lowest lethal dose
MED	Minimum effective dose
MTD	Maximum tolerated dose
NOEC	No-observed-effect concentration
ppm	Parts per million
RNA	Ribonucleic acid
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase

LIST OF ABBREVIATIONS (cont.)

TNT	Trinitrotoluene
TPA	Tetradecanoylphorbate acetate
TWA	Time-weighted average
UDS	Unscheduled DNA synthesis

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Table 1-1 lists the DNTs, including synonyms, that are reviewed in this document. The CAS numbers, molecular weight, empirical formula and structures of the DNT isomers are given in Table 1-2.

1.2. PHYSICAL AND CHEMICAL PROPERTIES

DNTs are solids at room temperature, are not very soluble in water, have intermediate octanol/water partition coefficients and have relatively low vapor pressure. More complete chemical and physical properties of the DNTs are given in Table 1-3.

1.3. PRODUCTION DATA

DNTs are made by nitrating toluene with a mixture of sulfuric and nitric acid. Dinitration of toluene with a typical sulfuric/nitric acid mixture yields the following mixture of isomers: 78 wt% 2,4-DNT, 19 wt% 2,6-DNT, 2.5 wt% 3,4-DNT, 1 wt% 2,3-DNT and 0.5 wt% 2,5-DNT (Dunlap, 1981). To make a single isomer, the nitration is done in steps; for example, to make 2,4-DNT, nitration is stopped after the mononitrotoluene is made; pure p-nitrotoluene is obtained by crystallization and then nitration to yield only the 2,4-DNT isomer is carried out (Hoff, 1983). Nitration of m-nitrotoluene yields primarily 3,4-DNT and small amounts of 2,3-DNT and 2,5-DNT, while nitration of o-nitrotoluene gives 2,5-DNT and 2,6-DNT (Dunlap, 1981). Nitration may be done as either a batch or continuous-flow process (Albright, 1981). USITC (1983) reported that 719,889,000 pounds of a mixture of 2,4- and 2,6-DNT was produced in the United States in 1982. Assuming that this mixture is 78 wt% 1,4-DNT and 19 wt% 2,6-DNT (Dunlap, 1981), then $5.69x10^8$ pounds were 2,4-DNT and $1.37x10^8$ pounds were 2,6-DNT. Production data from the 1977 U.S. EPA TSCA inventory appear in Table 1-4.

TABLE 1-1

Synonyms for Selected Dinitrotoluenes

Common Name	Synonyms
Dinitrotoluene	methyldinitrobenzene dinitrophenylmethane DNT
2,3-Dinitrotoluene	1-methyl-2,3-dinitrobenzene 2,3-DNT
2,4-Dinitrotoluene	1-methyl-2,4-dinitrobenzene o,p-dinitrotoluene 2,4-dinitrotoluene 2,4-DNT
2,5-Dinitrotoluene	2-methyl-1,4-dinitrobenzene 2,5-DNT
2,6-Dinitrotoluene	2-methyl-1,3-dinitrobenzene 2,6-DNT
3,4-Dinitrotoluene	4-methyl-1,2-dinitrobenzene 3,4-DNT

TABLE 1-2

CAS Registry Numbers, Molecular Weight, Empirical Formula and Structures of Selected DNTs

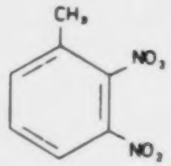
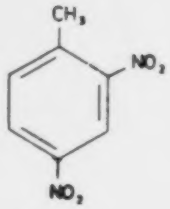
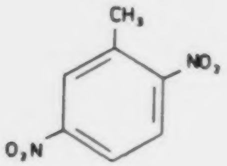
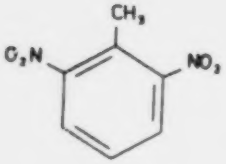
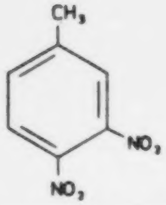
Isomer(s)	CAS Number	Molecular Weight	Empirical Formula	Structure
DNT	25321-14-6	182.14	$C_6H_3CH_3(NO_2)_2$	mixture of isomers
2,3-DNT	602-01-7	182.14	$C_6H_3CH_3(NO_2)_2$	
2,4-DNT	121-14-2	182.14	$C_6H_3CH_3(NO_2)_2$	
2,5-DNT	619-15-8	182.14	$C_6H_3CH_3(NO_2)_2$	
2,6-DNT	606-20-2	182.14	$C_6H_3CH_3(NO_2)_2$	
3,4-DNT	610-39-9	182.14	$C_6H_3CH_3(NO_2)_2$	

TABLE 1-3

Physical and Chemical Properties of Selected DNTs

Property	2,3-DNT	2,4-DNT	2,5-DNT	2,6-DNT	3,4-DNT
Melting point, °C	59-61[a]	67-70[b]	52.5[c]	64-66[a]	54-57[b]; 58.3[c]
Boiling point, °C (1 atm)	NR	300 (slight decomposition)[a]	285[d]	NR	NR
Density, g/cm³	NR	1.521 (d_4^{15})[e]	1.282 at 111°C[c]	1.2833 at 111°C[c]	1.2594 at 111°C[c]
Vapor pressure, mm Hg	NR	1.1×10^{-4} at 20°C[f]	NR	3.5×10^{-4} at 20°C[g]	NR
Water solubility	NR	0.03 g/100 g H_2O at 22°C (300 ppm)[e]	NR	NR	NR
Log K_{ow}	1.98[h] (calculated)	1.98[h] (measured)	1.98[h] (calculated)	1.72[h] (calculated)	2.28[h] (calculated)
Henry's Law constant at 20°C, atm-m³/mol	not calculated[i]	8.79×10^{-8}[j]	not calculated	not calculated[i]	not calculated[i]
Physical appearance at room remperature	NR	yellow needles[a]	NR	NR	yellow needles[c]

[a] Verschueren, 1983

[b] Aldrich, 1984

[c] Weast, 1973

[d] Maksimov, 1968

[e] Dunlap, 1981

[f] Spanggord et al., 1980a

[g] Pella, 1977

[h] U.S. EPA, 1984a

[i] To calculate Henry's Law Constant, the vapor pressure and water solubility must be known

[j] Calculated from vapor pressure and water solubility

NR = Not reported

TABLE 1-4

Production Data for Selected DNTs[a]

Isomer(s)	Producer	Production (pounds/year)
DNT	Mobay Chemical Corp., New Martinsville, WV	NA
	Mobay Chemical Corp., Baytown, TX	NA
	Union Carbide Corp., Institute, WV	NA
	Rubicon Chemicals Inc., Geismar, LA	NA
2,3-DNT	Air Products and Chemicals, Inc., Pasadena, TX	1,000,000-10,000,000
	E.I. Du Pont de Nemours and Company, Deepwater, NJ	NA
2,4-DNT[b]	Uniroyal, Inc., Joliet, IL	NA
	Allied Chemical Corp., Moundsville, WV	NA
	Air Products and Chemicals, Inc., Pasadena, TX	100,000,000-500,000,000
	E.I. Du Pont de Nemours and Company, Deepwater, NJ	NA
2,5-DNT	Air Products and Chemicals, Inc., Pasadena, TX	1,000 000-10,000,000
	E.I. Du Pont de Nemours and Company, Deepwater, NJ	NA
2,6-DNT	Allied Chemical Corp., Moundsville, WV	NA
	Air Products and Chemicals, Inc., Pasadena, TX	50,000,000-100,000,000
	E.I. Du Pont de Nemours and Company, Deepwater, NJ	NA

TABLE 1-4 (cont.)

Isomer(s)	Producer	Production (pounds/year)
3,4-DNT	Air Products and Chemicals, Inc., Pasadena, TX	1,000,000-10,000,000
	E.I. Du Pont de Nemours and Company, Deepwater, NJ	NA

[a] Source: U.S. EPA, 1977

[b] SRI International (1985) lists the current 2,4-DNT producers as: Air Products and Chemicals., Inc., Pasadena, TX; E.I. Du Pont de Nemours and Company, Deepwater, NJ; ICI Americas, Inc., Geismar, LA; and Olin Corp., Moundsville, WV.

NA = Not available

1.4. USE

DNTs are used in organic synthesis, dyes, explosives (Hawley, 1981) and as a propellant additive (Sears and Touchette, 1982). A major use of 2,4-DNT is the making of 2,4-diaminotoluene (Hoff, 1983).

1.5. SUMMARY

The 2,3-, 2,4-, 2,5-, 2,6- and 3,4- isomers of DNT are reviewed in this document. All five isomers are solid at room temperature (Aldrich, 1984; Verschueren, 1983; Weast, 1973). The water solubility and vapor pressure of one of the more common isomers, 2,4-DNT, is 300 ppm at 22°C (Dunlap, 1981) and 1.1×10^{-4} mm Hg at 20°C (Spanggord et al., 1980a), respectively. From the 1977 TSCA inventory data (U.S. EPA, 1977), it appears that 2,4-DNT has the highest production volume, followed by 2,6-DNT. Assuming that a commercially-produced mixture of 2,4- and 2,6-DNT is 79% 2,4-DNT and 19% 2,6-DNT (Dunlap, 1981), then $\sim 5.69 \times 10^{8}$ and 1.37×10^{8} pounds of 2,4- and 2,6-DNT, respectively, were produced in the United States in 1982 (USITC, 1983). DNTs are used in organic synthesis, dyes, explosives (Hawley, 1981) and as a propellant additive (Sears and Touchette, 1982). A major use of 2,4-DNT is the making of 2,4-diaminotoluene (Hoff, 1983).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

2.1.1. Partitioning. The measured log K_{ow} value for 2,4-DNT is 1.98 (U.S. EPA, 1984a). Calculated log K_{ow} values for the other isomers range from 1.72-2.28 (see Table 1-3). These intermediate log K_{ow} values suggest that DNTs in water will have a slight tendency to sorb to sediments, suspended solids and biota. This is supported by experimental data by Spanggord et al. (1980b) that indicated biosorption and sediment sorption would not be significant in the environment. Volatilization of DNTs from water probably will not be a significant transport mechanism. The Henry's Law constant calculated for 2,4-DNT from the solubility and vapor pressure data in Table 1-3 is 8.79×10^{-8} atm•m^3/mol (0.0688 torr/m). Henry's Law constant values $<3 \times 10^{-7}$ atm•m^3/mol indicate that the chemical is essentially nonvolatile (Lyman et al., 1982). Therefore, 2,4-DNT, and probably all the DNT isomers, may be considered to be essentially nonvolatile in water. 2,4-DNT, for example, is estimated to have a volatilization half-life of several hundred days from ponds, lakes and rivers (Smith and Bomberger, 1981). Spanggord et al. (1980b) estimated the volatilization rate constant for 2,4-DNT from a body of water as 6.6×10^{-5} hour^{-1} ($t_{1/2}$ = 438 days). Davis et al. (1983) studied the fate of 2,6-DNT, including volatilization, in a model waste stabilization pond. Influent 2,6-DNT concentration was 1 mg/ℓ, temperature was 23±2°C, and the detention time in the pond was 12 days. Nine other organic compounds and powdered dry milk were also in the influent. The fate of 2,6-DNT (as a percentage of the total dose) was: 0.3% volatile loss, 92.2% degradation, 3.6% sedimentation, 1.2% water column residuals and 2.7% loss in effluent.

volatilization half-life for 2,6-DNT from this model pond was 200 hours. Davis et al. (1983) also measured a 2,6-DNT BCF of 5225 for the algal biomass in their model pond.

2.1.2. **Photolysis.** Spanggord et al. (1980b) studied sunlight photolysis of 1.0 mg/ℓ 2,4-DNT in distilled and natural waters. Half-lives were 43 hours in distilled water and 2.7, 9.6 and 3.7 hours in river, bay and pond waters, respectively. The half-life for 2,6-DNT in river water exposed to sunlight was 12 minutes and was determined to be an indirect photoreaction (Zepp et al., 1984). Zepp et al. (1984) further reported that natural substances present in aquatic environments, such as humic substances, suspended sediments and algae, influence photolysis rates of pollutants by attenuation of sunlight or through indirect photoprocesses (sensitization of photoreactions). Thus photolysis may also be a significant degradation mechanism for the other DNTs.

2.1.3. Biodegradation.

2.1.3.1. **AEROBIC BIODEGRADATION** -- Isbister et al. (1980), in a review of the literature, concluded that 2,4-DNT degradation was rapid in the presence of large mixed populations of microorganisms under aerobic conditions. Liu et al. (1984), however, found that 2,4-DNT, as the sole carbon and energy source, did not degrade in an aerobic batch culture after 14 days of incubation. The 2,4-DNT concentration was from 5-25 mg/ℓ and the inoculum was municipal activated sludge. On the other hand, several studies indicate that 2,4-DNT rapidly biodegraded. Davis et al. (1981) found that 2,4-DNT degraded rapidly in 2 days in a batch culture containing glucose and inoculated with an industrial inocula The industrial seed contained four bacterial genera (Acintobacter, Alcaligenes, Flavobacterium and Pseudomonas) and one yeast (Rhodotorula). Municipal seed (conventional activated sludge organisms), however, was inhibited by 2,4-DNT concentrations as low as 10 mg/ℓ. In a Warburg respirometer, oxygen uptake was ~100 μℓ/150 minutes at an initial 2,4-DNT concentration of 100 mg/ℓ (Barth and Bunch, 1979). The inoculum was taken from a mixed culture acclimated to 300 mg/ℓ phenol. Spanggord et al. (1980b) found that natural surface water microorganisms could use 10 mg/ℓ 2,4-DNT as a sole carbon source. After a 2- to 3-day lag period, >90% of 10 mg/ℓ 2,4-DNT was transformed in aerated water in 6 days of incubation. This same culture was acclimated to grow on 120 mg/ℓ 2,4-DNT. Tabak et al. (1981) tested the biodegradability of 2,4-DNT and 2,6-DNT in a static screening test. Each flask contained the test chemical, 5 mg/ℓ yeast extract and settled domestic sewage as the inoculum. Results appear in Table 2-1. For some reason, the biodegradation rate decreased with each subculture. Tabak et al. (1981) suggested that either their subculture method resulted in a loss of synergistic activity exhibited by the original heterogenous population on the substrate or accumulated toxic by-products of metabolism retarded the inductive enzyme process.

Hallas and Alexander (1983) found that 2,6-DNT at an initial concentration of 100 mg/ℓ did not significantly degrade after 28 days incubation with raw municipal sewage. In another study, 2,6-DNT inhibited municipal sewage at concentrations >50 mg/ℓ (Davis et al., 1981). In the same study, industrial seed degraded 50 mg/ℓ 2,6-DNT to 25 mg/ℓ in 2 days, but after 7 days, 2,6-DNT concentration remained at 25 mg/ℓ. Matsui et al. (1975) concluded that DNT was resistant to degradation by activated sludge because it did not exert any BOD after 24 hours incubation.

TABLE 2-1

Results of 2,4-DNT and 2,6-DNT Static Screening Test*

Test Compound	Concentration of Test Compound (mg/ℓ)	Average of Three Test Flasks (Percent Biodegradation of Test Compound in 7 Days)			
		Original Culture	First Subculture	Second Subculture	Third Subculture
2,4-DNT	5	77	61	50	27
	10	50	49	44	23
2,6-DNT	5	82	55	47	29
	10	57	49	35	13

*Source: Tabak et al., 1981

Parrish (1977) screened 190 fungi representing 98 genera and found that only five organisms were able to transform 2,4-DNT at an initial concentration of 100 mg/ℓ. Biotransformation of 100 mg/ℓ 2,4-DNT by Mucrosporium sp. (fungus) yielded 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,2'-dinitro-4,4'-azoxytoluene, 4,4'-dinitro-2,2'-azoxytoluene and 4-acetamide-nitrotoluene (McCormick et al., 1978).

2.1.3.2. **ANAEROBIC BIODEGRADATION** -- Hallas and Alexander (1983) found that 2,4-DNT degraded slowly under anaerobic conditions. Raw municipal sewage was used as the inoculum and the 2,4-DNT concentration was 10 mg/ℓ. McCormick et al. (1976) found that under anaerobic conditions, cell-free extracts from the strict anaerobe Veillonella alkalescens first reduced the 4-nitro group on 2,4-DNT. In another study, 2,4-DNT was microbially transformed under anaerobic conditions by a mixed culture derived from activated sludge (Liu et al., 1984). Biotransformation products of 2,4-DNT were 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2-nitroso-4-nitrotoluene and 4-nitroso-2-nitrotoluene. However, the two nitroso compounds were unstable and could be detected only in the early stage of 2,4-DNT anaerobic biotransformation.

2.1.4. **Hydrolysis.** According to Lyman et al. (1982), aromatic nitro compounds are not susceptible to hydrolysis. Spanggord et al. (1980b) found that 2,4-DNT concentration did not change after 2 weeks incubation in sterile, natural water. Based on this evidence and because photolysis and biodegradation are relatively rapid, hydrolysis is not expected to be a significant fate process for the DNTs.

2.2. AIR

Since no information was found in the available literature about the fate of DNTs in the atmosphere, a computer estimate was done for each DNT isomer (U.S. EPA, 1985). The atmospheric half-life that was due to reaction with photochemically generated hydroxyl radical for each of the DNTs was estimated to be 8.0 hours at a hydroxyl radical concentration of $8x10^5$ molecules/cm^3.

2.3. SOIL

Ninety-five percent of DNT was removed from Rhine River water after infiltration through dune soil (Piet et al., 1981). Other information regarding the fate of DNTs in soil could not be located in the available literature. Since DNTs have been shown to biodegrade in both aerobic and anaerobic aquatic systems, they will probably also biodegrade in soil. Soil mobility of DNTs was estimated using an equation of Lyman et al. (1982) and log K_{ow} values of 1.72-2.28 (see Table 1-3). The estimated K_{oc} values range from 205-414. According to Kenaga (1980), K_{oc} values >1000 indicate that a compound is tightly bound to organic matter in the soil and is considered immobile. Compounds with K_{oc} values <100 are moderately to highly mobile. DNTs with K_{oc} values from 205-414 therefore might be considered slightly mobile in soil.

2.4. SUMMARY

DNTs released to water will remain primarily in the water column. The measured log K_{ow} value for 2,4-DNT is 1.98 (U.S. EPA, 1984a). Calculated log K_{ow} values for the other isomers range from 1.72-2.28 (U.S. EPA, 1984a). These intermediate log K_{ow} values suggest that DNTs in water will have a slight tendency to sorb to sediments, suspended solids and biota. This is supported by experimental data by Spanggord et al. (1980b), which indicated that biosorption and sediment sorption would not be significant in the environment. Volatilization of DNTs from water will not be a significant transport mechanism. For example, Spanggord et al. (1980b) estimated the volatilization half-life of 2,4-DNT from a body of water as 438 days.

In water, photolysis and biodegradation will be the primary degradation mechanisms. Photolysis half-lives for 1.0 mg/ℓ 2,4-DNT were 43 hours in distilled water and 2.7, 9.6 and 3.7 hours in river, bay and pond waters, respectively (Spanggord et al., 1980b). Biodegradation rates found in the literature varied widely, probably because of different inocula, acclimation schedules (or lack of) and DNT concentrations. The latter point is important because DNT is inhibitory at relatively low concentrations. For example, 2,4-DNT at 10 mg/ℓ was inhibitory to municipal activated sludge organisms (Davis et al., 1981). Spanggord et al. (1980b) found that natural surface water microorganisms could use 10 mg/ℓ 2,4-DNT as a sole carbon source. After a 2- to 3-day lag period, >90% of 10 mg/ℓ 2,4-DNT was transformed in aerated water in 6 days of incubation. This same culture was acclimated to grow on 120 mg/ℓ 2,4-DNT. Hydrolysis will probably not be an important fate process for DNTs. For example, 2,4-DNT concentration did not change after 2 weeks in sterile, natural water (Spanggord et al., 1980b).

Bailey (1982) estimated a half-life of 11.0 hours for 2,4-DNT in natural waters. Zoeteman et al. (1980) estimated the half-life of 2,4-DNT as 1.7 days in the Rhine River. The other DNT isomers will probably behave similarly.

The atmospheric half-life for each of the DNT isomers was estimated as 8.0 hours due to reaction with photochemically produced hydroxyl radicals (U.S. EPA, 1985). In the soil, DNT will be slightly mobile with K_{oc} values estimated from 205-414 (Lyman et al., 1982). DNTs may biodegrade in soils based on comparison with aquatic biodegradation studies.

3. EXPOSURE

3.1. WATER

Table 3-1 lists monitoring data for DNTs in effluents, surface waters and drinking water. Because of insufficient data on DNT concentrations in drinking water, an average daily intake could not be estimated.

3.2. FOOD

Pertinent data regarding the level of DNTs in food could not be located in the available literature as cited in the Appendix.

3.3. INHALATION

Air sampling for DNT was performed in October, 1979 at the Du Pont Chambers worksite in Deepwater, NJ (Fannick, 1981), where levels ranged from undetectable to 0.042 mg/m^3 (OSHA standard of 1.5 mg/m^3). Another occupational survey was done at the Olin Chemical Company in Brandenburg, KY, on September 26 and 27, 1979, and January 14-18, 1980, for DNT. DNT concentrations in the air ranged from 0.013-0.42 mg/m^3 during the initial survey and from undetectable to 0.10 mg/m^3 during the follow-up survey (Ahrenholz, 1980). If the average worker breathed 10 m^3 of air during an 8-hour work shift at a DNT concentration of 0.10 mg/m^3, and an absorption efficiency of 50% is assumed, then he would be exposed to 0.5 mg of DNT.

3.4. DERMAL

Pertinent data regarding dermal exposure to DNT could not be located in the available literature as cited in the Appendix.

3.5. SUMMARY

DNTs are commonly found in wastewater from TNT production. For example, 2,4-DNT and 2,6-DNT average concentrations in wastewater from TNT production were 9.7 and <0.3 mg/ℓ, respectively (Spanggord and Suta, 1982). DNT was detected in some samples from the Delaware River basin at >1 μg/ℓ

TABLE 3-1

Monitoring Data for DNTs in Water

Chemical	Sample Type and/or Location	Concentration	Reference
2,6-DNT	raw wastewater from a textile plant	50 mg/m^3	Rawlings and Samfield, 1978
2,3-DNT	wastewater from 2,4,6-trinitrotoluene production (average concentrations)	0.173 mg/ℓ	Spanggord and Suta, 1982
2,4-DNT		9.731 mg/ℓ	
2,5-DNT		0.215 mg/ℓ	
2,6-DNT		4.292 mg/ℓ	
3,4-DNT		0.319 mg/ℓ	
DNT	Rhine River water (The Netherlands)	1 μg/ℓ	Piet and Morra, 1983
2,3-DNT	Dokai Bay, Japan	ND-0.412 μg/ℓ	Hashimoto et al., 1982
2,4-DNT		ND-206 μg/ℓ	
2,6-DNT		ND-14.8 μg/ℓ	
DNT	two United States river basins (locations not specified)	>1 μg/ℓ	Ewing et al., 1977
DNT	Delaware River basin; dinitrotoluene was detected in 3% of the base extracted samples	>1 μg/ℓ	DeWalle and Chian, 1978
2,4-DNT	Rhine River water (The Netherlands)	0.3 μg/ℓ	Zoeteman et al., 1980
2,4-DNT	drinking water (unspecified location)	NS	Kool et al., 1982
2,6-DNT		NS	
2,4-DNT	condensate wastewater from 2,4,6-trinitrotoluene manufacture	NS	Liu et al., 1983a

NS = Not specified; ND = not detected

(DeWalle and Chian, 1978). 2,4-DNT concentrations in Dokai Bay (Japan) ranged from undetectable to 206 μg/ℓ (Hashimoto et al., 1982). Because of insufficient data on DNT concentrations in drinking water and food, average daily intakes could not be calculated. DNT air concentrations in the workplace ranged from undetectable to 0.042 mg/m³ at the Du Pont Chambers worksite in Deepwater, NJ (Fannick, 1981) and at the Olin Chemical Company in Brandenburg, KY, from undetectable to 0.42 mg/m³ (Ahrenholz, 1980). Information regarding dermal exposure from DNTs could not be located in the available literature.

4. PHARMACOKINETICS

4.1. ABSORPTION

Limited data suggest that 2,4-DNT is readily absorbed through the skin and respiratory tract, although the extent of absorption has not been reported (U.S. EPA, 1980a). Percutaneous contact and inhalation are both considered industrially important routes of exposure for 2,4-DNT (U.S. EPA, 1980a).

Oral administration of ^{14}C-labeled DNTs to rats resulted in the absorption of 60-90% of the dose within 24 hours (Hodgson et al., 1977). The observed extent of absorption was as follows: 2,4-DNT = 3,4-DNT > 3,5-DNT = 2,5-DNT > 2,3-DNT = 2,6-DNT. Mori et al. (1978) observed that peak values for the concentration of radioactivity in systemic circulation of the rat occurred ~6 hours following oral administration of ^{3}H-2,4-DNT. Twenty-four hours after oral administration of 2,4-DNT, only 8-12% of the dose was absorbed in mice, while up to 85% was absorbed in monkeys, dogs, rabbits and rats (TSCA-ITC, 1981). Rickert et al. (1980) demonstrated that 2,4-DNT was able to cross the placenta of rats to an extent that concentrations of the compound in maternal and fetal tissues was very similar.

Although few direct absorption data are contained in the available literature, oral, inhalation and dermal absorption can be inferred from the toxicity and metabolism studies using DNT isomers (Sections 4.2. to 4.4.).

4.2. DISTRIBUTION

Studies using radiolabeled isomers of DNT have generally demonstrated that most of the compound is eliminated within 24 hours of administration and that distribution of radioactivity occurs in the liver, kidneys, skin and adipose tissue. Seven days after the oral administration of ^{3}H-2,4-DNT to Wistar rats, 0.4, 0.6 and 1.6% of the radioactivity remained in the liver, skin and adipose tissue, respectively (Mori et al., 1977). No more than 0.1% of the radioactivity was present in any other organ. In a related study, it was observed that the time courses of radioactivity in the blood and liver of rats dosed orally with [^{3}H]-2,4-DNT were similar, with peak levels occurring at 6 hours. Elimination of radioactivity from blood followed first-order kinetics which was mono-exponential with a half-life of ~22 hours reflecting a one-compartment model (Mori et al., 1978).

Tissue/plasma radioactivity ratios in rats orally administered several ring-labeled ^{14}C-DNT isomers indicated that the liver and kidneys retain some ^{14}C while other tissues do not (Hodgson et al., 1977). Rickert et al. (1983) observed that hepatic concentrations of radioactivity in rats occurred in two stages (for each compound) with the first peak occurring 1-2 hours and the second peak occurring 8-12 hours after an oral dose of ^{14}C-2,4-DNT or ^{14}C-2,6-DNT. The second peaks were probably resulting from enterohepatic recycling. The concentration of the 2,6-isomer was constantly greater than that of 2,4-DNT. The terminal half-life for the loss of radioactivity was estimated as 3.4-4.4 days for both isomers. Similar results in CD rats given 2,4-DNT were reported by Ellis et al. (1979). In both control rats and rats pretreated for up to 20 months with 2,4-DNT, the liver and kidneys contained the highest percentage of radioactivity 24 hours after a single oral dose.

There was no evidence of preferential uptake or retention of parent compound in various tissues in strain A mice given a range of oral or intraperitoneal doses of ^{3}H-2,4-DNT (Schut et al., 1982). In a related study, there was no accumulation in any tissue of 2,6-DNT given orally or by intraperitoneal injection in mice (Schut et al., 1983).

Lee et al. (1978) examined the distribution of ^{14}C-2,4-DNT in several species 24 hours after a single oral dose (Table 4-1). Aside from the gastrointestinal tract, dogs and monkeys appear to accumulate substantially more of the radioactivity, retaining ~3.6 and 2.2% of the dose, respectively. The highest tissue/plasma radioactivity ratio occurred in the livers of all species studied.

4.3. METABOLISM

Biotransformation of the DNTs, as evidenced by in vivo and in vitro studies, is rapid and extensive. Both hepatic and microfloral enzymes are primarily responsible for DNT metabolism. Incubation of DNT with various hepatic preparations (microsomal, cytosolic or postmitochondrial supernatant fractions) under nitrogen (anaerobic) yielded monoaminonitrotoluenes predominantly regardless of the isomer used or species studied (Decad et al., 1982; Dent et al., 1982; Kozuka et al., 1978; Mori et al., 1984b; Short et al., 1979). Reduction of the nitro group was most pronounced at the less sterically hindered position such that groups meta or para to the methyl group were metabolized more rapidly than ortho groups (Kozuka et al., 1978). One study reported the formation of a hydroxylamino derivative when 2,3-DNT was incubated with a rat liver homogenate (Kozuka et al., 1978). It also appears that the cytosol contains substantially more reductive capacity than microsomes since the rate of monoaminonitrotoluene formation in the cytosol was greater than in microsomes (Decad et al., 1982) and only the cytosol fraction from rats was able to further reduce the monoamino derivatives of 2,4-DNT to the well established carcinogen, 2,4-diaminotoluene (Mori et al., 1984b).

TABLE 4-1

Distribution of Radioactivity in Mammals 24 Hours After a Single Oral Dose of ^{14}C-2,4-DNT*

Site of Disposition	Percent of Administered Dose				
	Mouse (CD-1)	Rat	Rabbit	Dog	Monkey
Gastrointestinal tract plus contents	2.1	2.8	10.9	8.6	4.7
Whole blood	0.1	0.1	0.1	0.6	0.3
Spleen	<0.1	NR	<0.1	<0.1	<0.1
Liver	0.2	0.3	<0.3	1.1	0.7
Kidney	<0.1	<0.1	<0.1	0.2	<0.1
Brain	<0.1	<0.1	<0.1	<0.1	<0.1
Lung	<0.1	<0.1	<0.1	<0.1	<0.1
Skeletal muscle	0.1	0.3	0.2	1.6	1.2

*Source: Lee et al., 1978

NR = Not reported

When 2,4-DNT was incubated with rat hepatic microsomes or postmitochondrial supernatant in the presence of room air or oxygen, reduction of the nitro groups was depressed and 2,4-dinitrobenzyl alcohol was the primary metabolite (Decad et al., 1982; Dent et al., 1982). Addition of hepatic mixed function oxidase inducers to the incubation enhanced the formation of 2,4-dinitrobenzyl alcohol, while mixed function oxidase inhibitors reduced its production (Decad et al., 1982). Similar results were obtained using isolated hepatocytes from Fischer 344 rats; 2,4-dinitrobenzyl alcohol represented 75-80% of the total metabolites formed (Bond and Rickert, 1981). The production of this metabolite was enhanced by pretreatment of rats with Aroclor 1254 (6-fold), phenobarbital (3.5-fold) and 3-methylcholanthrene (3.5-fold), while additions of SKF-52-A or 7,8-benzoflavone to the incubation decreased 2,4-dinitrobenzyl alcohol formation. These results suggested that cytochrome P-450- and P-448-dependent enzymes are involved in the oxidative metabolism of 2,4-DNT to 2,4-dinitrobenzyl alcohol. Furthermore, it was observed that hepatocytes under conditions of reduced oxygen increased the level of reductive metabolism of 2,4-DNT relative to oxidation, although 2,4-dinitrobenzyl alcohol formation still predominated (Bond and Rickert, 1981).

In isolated perfused Fischer 344 rat livers, oxidative metabolism of 2,4- and 2,6-DNT to the corresponding dinitrobenzyl alcohols followed by the formation of glucuronide conjugates was the primary route of metabolism (Bond et al., 1981; Long and Rickert, 1982). In both studies, it was observed that males excreted 3.3- to 8.6-fold more of the glucuronides into the bile than into the perfusates, while females predominantly excreted the glucuronides into the perfusate, even though both sexes had equivalent bile flow rates and had the same capacity to form the conjugates. In bile duct-cannulated rats, Medinsky and Dent (1983) reported males excreted ~25% of the radioactivity from an oral dose of ^{14}C-2,4-DNT into the bile over a 36-hour period, while females excreted ~18% over the same period; 2,4-dinitrobenzyl alcohol glucuronide accounted for 90% of the radioactivity in the bile in males and females. Biliary excretion was essentially completed by 24 hours in males and 12 hours in females. Thus, in vitro and in vivo studies have demonstrated that the primary contribution of the liver to DNT metabolism is oxidation of the parent compound to its dinitrobenzyl alcohol followed by glucuronide conjugation. Reduction metabolism in the liver appears to be minimal.

Microorganisms from rats, mice and humans have the capacity to reduce DNT isomers under anaerobic conditions. Studies using cecal microflora isolated from Fischer 344 and Wistar rats and Swiss mice have demonstrated that DNT isomers are rapidly reduced first to a nitroso derivative followed by further reduction to hydroxylaminonitrotoluenes and monoaminonitrotoluenes (Dent et al., 1982; Guest et al., 1982; Mori et al., 1985). Incubation of both monoaminonitrotoluene metabolites of 2,4-DNT with microflora preparations led to the formation of 2,4-diaminotoluene (Dent et al., 1982; Mori et al., 1985). Microorganisms from human intestines and fecal samples were able to produce qualitatively similar metabolites, although the rate of reduction was somewhat slower than in mice and rats (Guest et al., 1982; Mori et al., 1984a). Cecal contents from rats reduced 2,4-DNT at a rate of 0.5-1.0 μmol/g contents/minute, while liver hepatocytes reduced only 3-7 mmol/g liver/minute (Guest et al., 1982).

In summary, in vitro studies of DNT metabolism indicate that reduction of DNT isomers occurs through the formation of nitroso, hydroxylaminonitro, and monoaminonitro derivatives and takes place primarily in the gut where microflora have 500- to 1000-fold greater reductive capacity than the liver under physiological conditions (Figure 4-1). The liver is able to promote oxidative metabolism of DNT isomers resulting in the production of dinitrobenzyl alcohols and their corresponding glucuronide conjugates.

These conjugates are excreted into the bile at a much faster rate in males compared with females. Hepatic reductive metabolism of DNTs is relatively minor (Figure 4-2).

Analysis of urinary DNT metabolites has supported the findings of *in vitro* studies that the primary metabolic pathways for DNT isomers involve the reduction of a nitro group to an amino group and the oxidation of the methyl group to an alcohol, while secondary pathways involve N-acetylation of the amino moiety and further oxidation of the alcohol to a benzoic acid derivative (Mori et al., 1981). In male and female Fischer 344 rats orally dosed with ^{14}C-2,6-DNT, three major metabolites, which make up 95% of the radioactivity, were present in the urine 72 hours after administration: 2,6-dinitrobenzoic acid, 2-amino-6-nitrobenzoic acid and 2,6-dinitrobenzyl alcohol glucuronide (Long and Rickert, 1982). Oral administration of ^{14}C-2,4-DNT to rats resulted in four major urinary metabolites: 4-acetylamino-2-nitrobenzoic acid, 2,4-dinitrobenzoic acid, 2-amino-4-nitrobenzoic acid and 2,4-dinitrobenzyl alcohol glucuronide; these metabolites accounted for >85% of the radioactivity excreted in urine (Medinsky and Dent, 1983; Rickert and Long, 1981). Other metabolites present in rat urine included 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-dinitrobenzyl alcohol, 2-amino-4-nitrobenzyl alcohol, 4-amino-2-nitrobenzyl alcohol, 4-acetylamino-2-nitrotoluene, 4-acetylamino-2-aminotoluene, 4-acetylamino-2-aminobenzoic acid and 2,4-diaminotoluene (Medinsky and Dent, 1983; Mori et al., 1981).

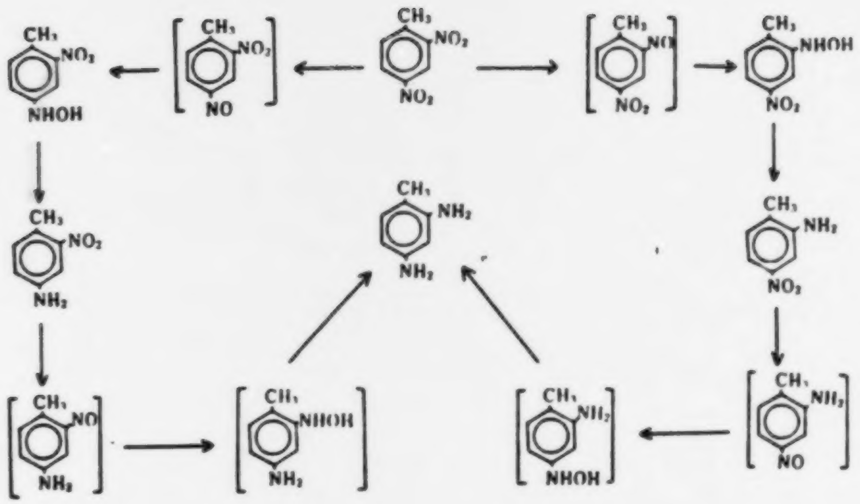
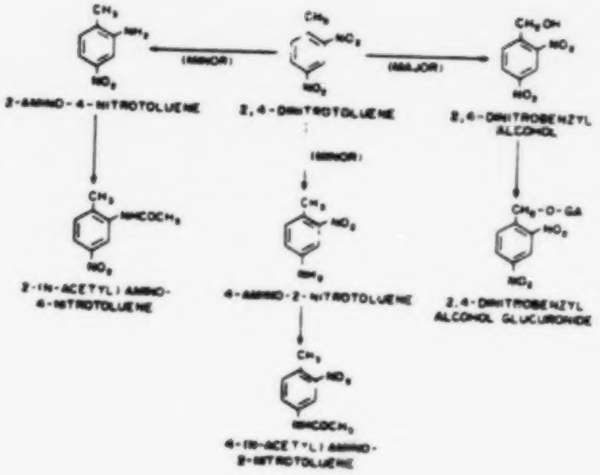

CH₃
NO₂
NHOH
CH₃
NO₂
NO
CH₃
NO₂
NO₂
CH₃
NO
NO₂
CH₃
NHOH
NO₂
CH₃
NO₂
NH₂
CH₃
NH₂
NH₂
CH₃
NH₂
NO₂
CH₃
NO
NH₂
CH₃
NHOH
NH₂
CH₃
NH₂
NHOH
CH₃
NH₂
NO

FIGURE 4-1

Proposed Pathway for the Anaerobic Metabolism of 2,4-DNT in Rat Intestinal Microflora

Source: Mori et al., 1985



CH₃
NH₂
NO₂
(MINOR)
CH₃
NO₂
NO₂
(MAJOR)
CH₂OH
NO₂
NO₂
2-AMINO-4-NITROTOLUENE
2,4-DINITROTOLUENE
2,4-DINITROBENZYL ALCOHOL
(MINOR)
CH₃
NHCOCH₃
NO₂
2-(N-ACETYL) AMINO-4-NITROTOLUENE
CH₃
NO₂
NH₂
4-AMINO-2-NITROTOLUENE
CH₂-O-GA
NO₂
NO₂
2,4-DINITROBENZYL ALCOHOL GLUCURONIDE
CH₃
NO₂
NHCOCH₃
4-(N-ACETYL) AMINO-2-NITROTOLUENE

FIGURE 4-2

Proposed Metabolic Pathway(s) for the Hepatic Metabolism of 2,4-DNT

Source: Bond and Rickert, 1981

Similar metabolites have also been found in the plasma and kidneys of rats given 2,4-DNT (Rickert and Long, 1980). The only major sex difference in the metabolism was that females excreted up to twice the amount of the most prominent metabolite, the glucuronide, compared with males (Medinsky and Dent, 1983; Rickert and Long, 1981).

In Strain A mice administered 2,4- or 2,6-DNT orally or intraperitoneally, no unchanged parent compound could be detected within 2 hours; the liver and small intestine were the major sites of metabolism (Schut et al., 1982, 1983). Since the lungs and blood contained small amounts of unchanged 2,4-DNT 1 hour after dosing, these tissues were not considered active sites of metabolism for 2,4-DNT (Schut et al., 1982). Metabolites in mouse urine 2 hours following intraperitoneal injection of 2,4-DNT included 2,4-dinitrobenzyl alcohol, 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene, while 8 hours following oral administration, these metabolites were detected along with 2-acetylamino-4-nitrotoluene, 2-amino-4-nitrobenzyl alcohol and 2,4-diaminotoluene (Schut et al., 1985).

Rhesus monkeys, beagle dogs, CD rats and New Zealand rabbits given 2,4-DNT (0.1 LD_{50}) orally had a similar pattern of urinary metabolites with 2,4-dinitrobenzyl alcohol glucuronide accounting for 20-33% of the dose and the aminonitrobenzyl alcohol glucuronides accounting for 8-19% of the administered dose (Lee et al., 1978). CD-1 mice were somewhat different as these glucuronides represented only 5.4% of the administered dose. Other urinary metabolites detected in these species included the aminonitrotoluenes, 2,4-diaminotoluene, 2,4-diaminobenzyl alcohol and 2,4-dinitrobenzoic acid (Lee et al., 1978).

It is evident from in vitro studies that both the liver and gut participate in DNT metabolism. Rickert et al. (1981) used germfree (axenic) Fischer 344 rats to examine the contribution of each of these metabolic sites. Although the major metabolites detected in normal rat urine were present in the urine from axenic rats, there was an 80-90% reduction in the amount of those metabolites undergoing both oxidation and reduction. Furthermore, in axenic males there was less than half the amount of covalent binding of radioactivity from ^{14}C-2,4-DNT to hepatic macromolecules compared with normal males. This suggests that microflora are necessary in the formation of proximate or ultimate electrophilic 2,4-DNT intermediates that participate in covalent binding. The importance of gut flora was demonstrated by Long and Rickert (1982) who reported that hepatic covalent binding in vivo is 40-50 times greater than that observed in studies using perfused livers, which did not involve microflora. In addition, Rickert et al. (1983) observed that no appreciable covalent binding occurred in the livers of male rats during the first 4 hours after 2,4- or 2,6-DNT administration (i.e., during the first pass through the liver) and that significant binding took place only after glucuronide derivatives appeared in the intestine. Covalent binding to hepatic RNA, DNA and protein was first observed 8 hours after dosing and peaked 12-24 hours after dosing (Rickert et al., 1983). A significantly greater amount of hepatic covalent binding occurred in males compared with females, which may be related to the relative greater amount of glucuronide metabolites transported in the bile of males compared with females (Long and Rickert, 1982; Medinsky and Dent, 1983).

A scheme proposed by Bond et al. (1981) explains the role of the liver and the gut in DNT metabolism as well as the observed sex differences in glucuronide excretion in the bile and urine, and hepatic covalent binding of

DNT-derived compound (Figure 4-3). This hypothesis may also account for the increased susceptibility of male rats to DNT-induced hepatocarcinogenesis (Section 5.1.). DNTs are rapidly absorbed from the gastrointestinal tract and metabolized in the liver primarily to dinitrobenzyl alcohol followed by conjugation with glucuronic acid to form dinitrobenzyl alcohol glucuronide. The glucuronide is then excreted predominantly into the bile in males or preferentially into the general circulation and excreted in the urine in females. Biliary glucuronide is transported to the intestine where it is hydrolyzed back to the alcohol by gut microflora. Additional metabolism by the microflora in the intestine results in the reduction of a nitro group to the corresponding aminonitrobenzyl alcohol which can undergo further metabolism in the gut or can be reabsorbed and transported back to the liver. Once in the liver, covalent binding to hepatic macromolecules or further activation to a product that can covalently bind occurs. Thus, sex differences in excretion patterns and covalent binding may reflect the amount of glucuronide reaching the microflora for further activation. Since females excrete less glucuronide into the bile, less covalent binding would occur.

Rickert et al. (1984) reported that workers exposed to DNT have urinary excretion patterns for 2,4-DNT that are similar to those observed in Fischer 344 rats. The major metabolites of 2,4-DNT detected in human urine were 2,4-dinitrobenzoic acid, 2,4-dinitrobenzyl alcohol glucuronide, 2-amino-4-nitrobenzoic acid and 4-acetylamino-2-nitrobenzoic acid. For 2,6-DNT, however, 2,6-dinitrobenzoic acid and 2,6-dinitrobenzyl alcohol glucuronide were the only metabolites detected in human urine, reflecting a lack of reductive metabolism for 2,6-DNT. These data were preliminary, however, and require confirmation.

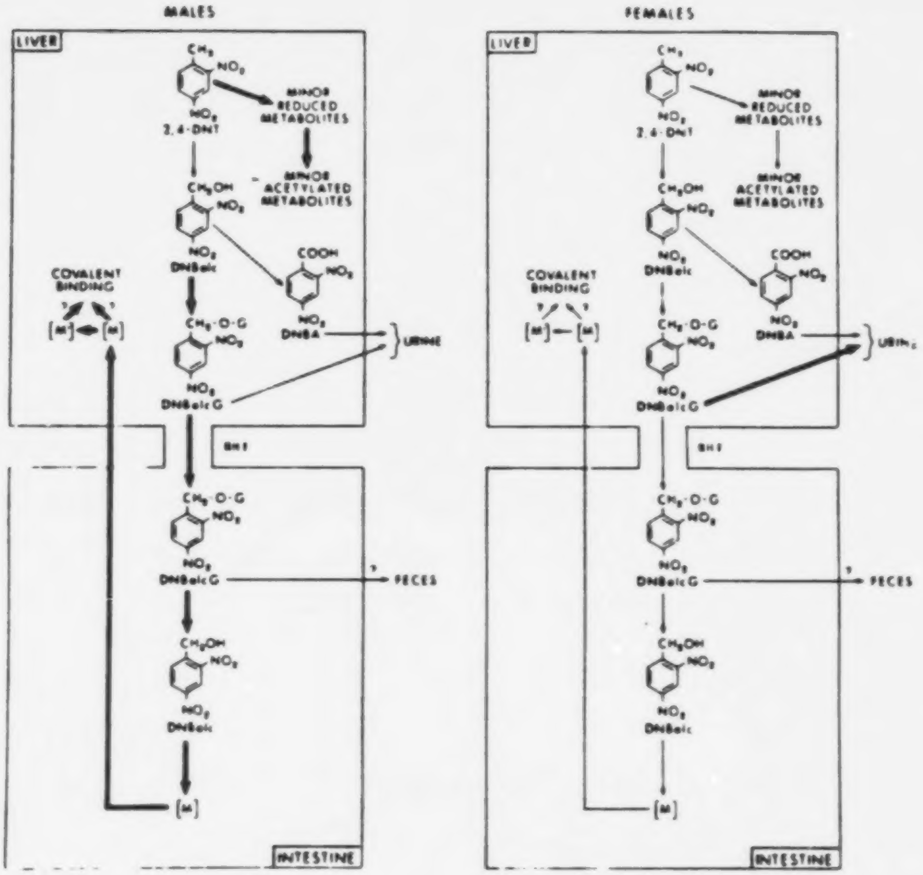

MALES
FEMALES
LIVER
CH3
NO2
2,4-DNT
MINOR REDUCED METABOLITES
MINOR ACETYLATED METABOLITES
CH2OH
DNBalc
COOH
DNBA
COVALENT BINDING
?
[M']
[M]
CH2-O-G
DNBalcG
URINE
FECES
INTESTINE

FIGURE 4-3

Schematic representation of a hypothesis to explain male/female differences in hepatic macromolecular covalent binding of radioactivity after administration of 2,4-^{14}C-DNT. Bolder arrows denote quantitatively more important pathways. M and M' denote possible active metabolite of DNBalc, DNBalc = dinitrobenzyl alcohol, DNBA = dinitrobenzoic acid, G = glucuronide.

Source: Bond et al., 1981

4.4. EXCRETION

Urine is the major route of 2,4-DNT excretion in dogs, monkeys and rabbits (Rickert et al., 1984). Urinary excretion also predominates in male and female Fischer 344 rats given 2,4- or 2,6-DNT. Long and Rickert (1982) reported that >50% of the administered dose of ^{14}C-2,6-DNT was excreted in the urine within 72 hours, while <20% was present in the feces. Urinary excretion was essentially complete within 24 hours, while fecal excretion persisted at 72 hours. Oral administration of ^{14}C-2,4-DNT produced a similar excretory pattern in rats. Single doses of 10, 35 and 100 mg/kg to Fischer 344 rats resulted in females excreting ~70, 82 and 50% of the administered dose in the urine, respectively, while male urine contained 50-55% of the administered radioactivity regardless of the dose level (Rickert and Long, 1981). Cumulative fecal excretion at 72 hours accounted for 10-30% of the dose in males and 10-17% in females. The excretion pattern in rats is dependent in part on enterohepatic recycling following biliary excretion since bile duct cannulation resulted in a marked decrease of ^{14}C excreted in the urine of rats treated with ^{14}C-2,4-DNT (Medinsky and Dent, 1983). In addition, intestinal microflora are important in DNT excretion since axenic rats given 35 mg/kg ^{14}C-2,4-DNT excreted a smaller percentage of the dose in the urine and feces than did conventional rats after 48 hours (Rickert et al., 1981).

A strain difference between Fischer 344 and Wistar rats is apparent from data reported by Mori et al. (1977, 1980) regarding the excretion of radioactivity from ^{3}H-2,4-DNT. In Wistar rats, fecal excretion accounted for 21% of the dose at 24 hours and 35% after 7 days, while only 13 and 20% of the dose was present in the urine 1 and 7 days, respectively, after dosing.

A similar pattern was observed in CD-1 and B6C3F1 mice, which excreted only 10% of a dose of 2,4-DNT in the urine and ~80% of the dose in the feces (Lee et al., 1978); however, Strain A mice almost exclusively excreted ^3H in urine following administration of ^3H-2,4-DNT orally or by intraperitoneal injection (Schut et al., 1982, 1983, 1985). Up to 90% of the dose given intraperitoneally appeared in the urine 4 hours after injection, while >50% of the dose was detected in the urine 8 hours after oral administration. Only 1.1-2.1% of the administered dose was recovered in the feces 8 hours after dosing, regardless of the route of exposure.

4.5. SUMMARY

The DNTs appear to be rapidly and extensively absorbed through the skin, respiratory tract and gastrointestinal tract in most species, although the available literature predominantly concerns oral absorption. Up to 90% of an oral dose of DNT was absorbed in rats within 24 hours (Hodgson et al., 1977) and maximum absorption occurred in the small intestine ~6 hours after administration (Mori et al., 1978). The observed extent of oral absorption for DNT isomers was 2,4- = 3,4- > 3,5- = 2,5- > 2,3- = 2,6-DNT (Hodgson et al., 1977). Mice were an exception since only 8-12% of an oral dose of 2,4-DNT was taken up by the intestine (TSCA-ITC, 1981).

Distribution studies have established that there is some accumulation of radioactivity from radiolabeled DNT isomers in the liver, kidneys, skin and adipose tissue (Mori et al., 1977; Hodgson et al., 1977; Ellis et al., 1979; Lee et al., 1978) in most species. Peak hepatic concentrations of radioactivity occurred 1-2 hours and 8-12 hours after oral administration of ^{14}C-2,4- or ^{14}C-2,6-DNT with radioactivity from the 2,6-isomer retained to a greater extent (Rickert et al., 1983). A similar pattern of distribution was also observed in rabbits, dogs and monkeys (Lee et al., 1978).

Metabolism of DNT isomers is extensive and occurs almost exclusively in the gut and liver of all species studied. In vitro studies have established that, under physiological conditions, oxidative metabolism predominates in the liver leading to the formation of dinitrobenzyl alcohol followed by conjugation to the corresponding glucuronide (Decad et al., 1982; Dent et al., 1982; Bond and Rickert, 1981; Bond et al., 1981; Long and Rickert, 1982). This glucuronide represents the major fraction of DNT metabolites excreted into the bile; males generally excreted more by this route than females (Medinsky and Dent, 1983).

Intestinal microflora from a number of species, including humans, are effective in reducing DNTs to monoaminonitrotoluenes and diaminonitrotoluenes (Dent et al., 1982; Guest et al., 1982; Mori et al., 1984a, 1985). Reductive DNT metabolism by gut microorganisms is estimated to occur at a rate ~1000 times greater than that observed in isolated hepatocytes (Guest et al., 1982).

Urinary DNT metabolites are composed chiefly of N-acetylaminonitrobenzoic acid, dinitrobenzoic acid, aminonitrobenzoic acid and dinitrobenzyl alcohol glucuronide derivatives (Long and Rickert, 1982; Medinsky and Dent, 1983; Rickert and Long, 1981) indicating that both reductive and oxidative metabolism occur in vivo (Mori et al., 1981). A major sex difference exists in the excretion of the glucuronide, the most common urinary metabolite; up to twice as much of this metabolite is found in female urine (Medinsky and Dent, 1983; Rickert and Long, 1981). Axenic rats were used to demonstrate the importance of gut microflora to the formation of metabolites undergoing oxidation and reduction (Rickert et al., 1981), while other studies reported that intestinal metabolism is related to the extent of DNT-derived compounds covalently binding to liver macromolecules (Rickert et al., 1981; Long and

Rickert, 1982; Rickert et al., 1983). Covalent binding of DNT-derived compounds to hepatic macromolecules is consistently more prevalent in males and may be associated with the greater concentrations of glucuronide metabolites transported in the bile of males compared with females (Long and Rickert, 1982; Medinsky and Dent, 1983).

A hypothesis presented by Bond et al. (1981) explains the general metabolic scheme for the DNT isomers. The compound initially is metabolized to a nitrobenzyl alcohol glucuronide in the liver and transported preferentially in the bile (males) or in the general circulation and urine (females). Once in the intestine, the glucuronide moiety is hydrolyzed and the resulting alcohol is reduced to a monoaminonitrotoluene, which is reabsorbed and returned to the liver. Covalent binding to DNA, RNA or protein or further transformation to a reactive electrophile then occurs within the liver. This enterohepatic scheme is able to reflect results obtained in both in vivo and in vitro studies and can reconcile the sex differences observed in DNT metabolism.

Preliminary data indicate that human DNT metabolism is similar to that observed in rats, at least for 2,4-DNT (Rickert et al., 1984).

Excretion of DNT isomers occurs almost exclusively through urine and feces although the relative contribution of each route is species and strain dependent. In most species, urinary excretion predominates with 50-80% of the dose eliminated in the urine within 24 hours, while the feces contained 10-30% of the administered dose after 72 hours (Rickert et al., 1984; Long and Rickert, 1982; Rickert and Long, 1981).

5. EFFECTS

5.1. CARCINOGENICITY

Several studies have investigated the carcinogenicity of technical grade DNT (a mixed isomer) and 2,4-DNT. In an NCI (1978) bioassay, groups of 50 male and 50 female Fischer 344 rats were administered 2,4-DNT in the diet at concentrations of 80 and 200 ppm for 78 weeks. At 78 weeks, five males and five females from each group were sacrificed. The remaining rats were killed after a 26-week observation period. Separate control groups were maintained for the high- and low-dose control groups and contained 25 and 50 rats/sex, respectively. All rats that died, were killed when moribund, or were sacrificed at the interim kill and at the end of the bioassay, were necropsied and subjected to a thorough histological examination. A few rats that were cannibalized or in an advanced state of autolysis were excluded from histological analysis. The survival rate for the two treatment groups did not differ significantly from their respective controls, with 52-64% of the males and 48-62% of the females surviving until the end of the study. As indicated in Table 5-1, a statistically significant increased incidence of fibromas of subcutaneous tissue of the skin occurred in both high- and low-dose males, while a significant increase in fibroadenomas of the mammary gland was observed in high-dose females. These benign lesions were the only tumors reported to have incidences significantly greater than the respective control groups in both male and female rats.

In the same study (NCI, 1978), groups of 50 male and 50 female B6C3F1 mice were fed 2,4-DNT at dietary concentrations of 80 and 400 ppm for 78 weeks and observed for an additional 13 weeks. Control groups containing 50 mice/sex for each dose group were maintained on the basal diet for 91 weeks.

TABLE 5-1

Incidence of Tumors in Fischer 344 Rats Treated with 2,4-DNT (purity >95%) in the Diet for 78 Weeks and Observed for an Additional 26 Weeks[a]

Sex	Concentration (ppm)	Target Organ	Tumor Type	Tumor Incidence (p value)
M	0 (low dose control)	subcutaneous tissue/skin	fibroma	0/46
	80	subcutaneous tissue/skin	fibroma	7/49 (p=0.008)[b]
	0 (high dose control)	subcutaneous tissue/skin	fibroma	0/25
	200	subcutaneous tissue/skin	fibroma	13/49 (p=0.003)[b]
F	0 (low dose control)	mammary gland	fibroadenoma	9/48
	80	mammary gland	fibroadenoma	12/49
	0 (high dose control)	mammary gland	fibroadenoma	4/23
	200	mammary gland	fibroadenoma	23/50 (p=0.016)[b]

TABLE 5-1 (cont.)

QUALITY OF EVIDENCE

Strengths of Study:	2,4-DNT was administered by a relevant route at two dose levels to both sexes of two species and encompassed the lifespan of the rats and 91 weeks for mice. Sufficient numbers of animals were used and survived for the detection of late-developing tumors. Doses were selected on the basis of a depression in mean group body weight. Histological examination was extensive. Adequate statistical analyses were performed.
Weakness of Study:	Different numbers of rats used in high dose and low dose control groups. Compound was not as pure as in other studies; other DNT isomers may be present. Interim kill for unspecified reason. The results of histological examination of rats killed at 78 weeks was not reported separately.
Overall Adequacy:	Adequate
Comments:	Mice data were negative for all tissues in both sexes at all dose levels and are not included in this table.

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[a] Source: NCI, 1978

[b] Significantly different from respective control by Fischer's Exact test.

Survival rates were similar for treated and control mice of both sexes, ranging between 74 and 90% for males and 70-84% for females. There was no evidence of a significant association between tumor incidence and dietary administration of 2,4-DNT in any tissues examined in these mice.

Another bioassay of practical grade 2,4-DNT for possible carcinogenicity was conducted by Lee et al. (1978) using CD rats (Charles River Breeding Laboratory, Wilmington, MA). The high dose, with 2,4-DNT intake of 34.0 mg/kg/day in male rats and 45.0 mg/kg/day in female rats, was quite toxic, causing decreased weight gain and shortened lifespan. Target organs included the blood (toxic anemia), liver (hepatocellular carcinoma), testes (aspermatogenesis) and connective tissue in male rats (fibromas), and the mammary tissue in female rats (fibroadenomas). No specific effects were seen on the reproductive process, on chromosomes, or on the metabolism of 2,4-DNT. The middle dose, with 2,4-DNT intake of 3.90 mg/kg/day in male rats and 5.10 mg/kg/day in female rats, was somewhat toxic. It caused similar effects in some, more susceptible, individual rats. The low dose, with 2,4-DNT intake of 0.57 and 0.71 mg/kg/day in male and female rats, respectively, had no apparent toxic effects. The carcinogenicity results for male and female rats are summarized in Tables 5-2 and 5-3, respectively.

Ellis et al. (1979) exposed groups of 38 male and 38 female CD (Sprague-Dawley) rats to dietary concentrations of 0, 15, 100 and 700 ppm 2,4-DNT for up to 24 months. Rats appearing moribund or those suffering from a lesion or serious injury were immediately sacrificed and necropsied. All rats that were sacrificed or found dead were given comprehensive histopathological examinations. Unscheduled deaths for both sexes began after 12 months of treatment and increased until the end of the study. Cumulative deaths reached 100% (high dose), 75% (middle dose) and 55% (low dose and controls) for male rats and 97% (high dose), 65% (middle dose) and 60% (low dose and

TABLE 5-2

Separate Tumor Incidence for All Age Groups of Female Rats Fed 2,4-DNT[a]

	Control (0 ppm) (0 mg/kg/day)	0.0015% (15 ppm) (0.71 mg/kg/day)	0.01% (100 ppm) (5.1 mg/kg/day)	0.07% (700 ppm) (45 mg/kg/day)
Liver[b]	0/31	3/43 (NS)	3/35 (NS)	30/42[c] ($p=3.96\times10^{-4}$)
Mammary gland tumor[d]	11/31	12/43 (NS)	18/35 (NS)	34/43[c] ($p=1.75\times10^{-4}$)
Combined mammary gland and liver tumor[e]	11/31	13/43 (NS)	18/35 (NS)	35/43[c] ($p=7.01\times10^{-5}$)

[a] Source: Lee et al., 1978

[b] Number of neoplastic nodule or hepatocellular carcinoma animals/number of animals in which livers were examined.

[c] The total number of animals examined microscopically for mammary gland tumors was 43. One animal of these 43 rats was missing liver tissue, i.e., livers examined were 42. However, the animal that was missing liver tissue had a mammary gland tumor, so it was counted as an animal having a tumor. Therefore, the total number of animals examined 0.07% dose was 43.

[d] Number of animals with either adenoma, fibroadenoma, fibroma or adenocarcinoma of the mammary gland/number of animals in which mammary gland tissues were examined.

[e] The number of animals that had either liver or mammary gland tumors or both/number of animals in which the liver and mammary glands were examined.

TABLE 5-3

Summary of the Male Rats with Tumors After Being Fed 2,4-Dinitrotoluene for 24 Months*

Dose (mg/kg/day)	Mammary Tumor/Total	Percent
0	1/37	3
0.57	0/37	0
3.90	0/29	0
34.0	17/23	74

*Source: Lee et al., 1978

controls) for females. Nevertheless, histopathology data obtained from these rats were useful for calculating tumor incidences. As reported in Table 5-4, there was an increased incidence of hepatocellular carcinoma in high-dose male and female rats, although statistical significance ($p<0.05$) was found only in females. Mammary tumors were prevalent in all female dose groups; benign lesions (fibroadenomas, adenomas, and fibromas) predominated. Statistically significant greater incidence of these mammary gland lesions was observed only for the high-dose group. The frequency of malignant tumors, specifically adenocarcinoma-carcinoma was consistently low in all treatment groups.

In another part of this study, groups of 58 male and 58 female CD-1 mice were maintained for 24 months on a diet containing 0, 100, 700 or 5000 ppm 2,4-DNT. The basic protocol for this segment of the study was identical to that used with rats, as explained above. Because most of the high-dose mice died before 12 months, this group was not considered representative of a lifetime exposure and was excluded from further analysis. Approximately 70% of the control and low-dose and 85% of the middle-dose males and 80% of the control, low- and middle-dose females died before scheduled sacrifice. As with rats, since these deaths occurred after 12 months these mice were used in calculating tumor incidences. There was no evidence of treatment-related increase in tumor frequency in any tissue in female mice. Liver tumors described as most probably being hepatomas, were observed in all dose groups in male mice, although there was no significant difference between the groups (Table 5-5). The most noteworthy observation in the males was the very high incidence of renal tumors including cystic papillary adenomas, solid renal cell carcinomas and cystic papillary carcinomas seen in the 700 ppm dose group. Low-dose males contained similar lesions but the incidence was not significantly different compared with controls (see Table 5-5).

TABLE 5-4

Incidence of Tumors in CD Rats Treated with 2,4-DNT (purity >98%) in the Diet for 24 Months[a]

Sex	Concentration (ppm)	Target Organ	Tumor Type[b]	Tumor Incidence[c] (p value)
M	0	liver	neoplastic nodule	1/25
			hepatocellular carcinoma	1/25
			neoplastic nodule or hepatocellular carcinoma	2/25
	15		neoplastic nodule	0/28
			hepatocellular carcinoma	2/28
			neoplastic nodule or hepatocellular carcinoma	2/28
	100		neoplastic nodule	0/19
			hepatocellular carcinoma	2/19
			neoplastic nodule or hepatocellular carcinoma	2/19
	700		neoplastic nodule	2/30
			hepatocellular carcinoma	6/30 (p=0.08)[d]
			neoplastic nodule or hepatocellular carcinoma	8/30 (p=0.07)[d]
F	0	liver	neoplastic nodule	0/23
	15		neoplastic nodule	3/35
			hepatocellular carcinoma	0/35
			neoplastic nodule or hepatocellular carcinoma	3/35
F	100	liver	neoplastic nodule	2/27
			hepatocellular carcinoma	1/27
			neoplastic nodule or hepatocellular carcinoma	3/27
	700		neoplastic nodule	5/35 (p=0.07)[d]
			hepatocellular carcinoma	19/35 (p=4.3x10^{-}[illegible])[d]
			neoplastic nodule or hepatocellular carcinoma	23/35 (p=9.5x10^{-}[illegible])[d]

TABLE 5-4 (cont.)

Sex	Concentration (ppm)	Target Organ	Tumor Type[b]	Tumor Incidence[c] (p value)
F	0	mammary gland	benign	10/23
			malignant	3/23
			benign or malignant	11/23
	15		benign	9/35
			malignant	3/35
			benign or malignant	11/35
F	100	mammary gland	benign	16/27
			malignant	3/27
			benign or malignant	17/27
	700		benign	34/35 ($p=4\times10^{-6}$)[d]
			malignant	0/35
			benign or malignant	34/35 ($p=1.5\times10^{-6}$)[d]
F	0	mammary/ liver	all types	11/23
	15			12/35
	100			17/27
	700			34/35 ($p=1.5\times10^{-5}$)[d]

TABLE 5-4 (cont.)

QUALITY OF EVIDENCE

Strengths of Study: 2,4-DNT was administered by a relevant route at three dose levels spaced at equal logarithmic intervals to both males and females. The experiment was designed to last the entire lifespan of the animals. Gross examinations and histopathological analyses were extensive.

Weakness of Study: Analysis of the test compound detected ~2% 2,6-DNT, which may affect tumor formation. Few animals survived the intended length of the study (control group survival rate was only 40-45%).

Overall Adequacy: Adequate

Comments: Rats that died before 12 months were excluded from this table.

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[a] Source: Ellis et al., 1979

[b] benign = adenoma, fibroadenoma and fibroma; malignant = adenocarcinoma-carcinoma.

[c] Many animals died before 24 months. They were nevertheless included in the tumor incidence data.

[d] Fischer's Exact test performed at SRC.

TABLE 5-5

Incidence of Tumors in Male CD-1 Mice Treated with 2,4-DNT (purity >98%) in the Diet for 24 Months[a]

Concentration (ppm)	Target Organ	Tumor Type[b]	Tumor Incidence[c] (p value)
0	liver	not specified	7/20
100			9/21
700			9/17
0	kidney	benign	0/20
		malignant	0/20
		benign or malignant	0/20
100		benign	2/21
		malignant	2/21
		benign or malignant	4/21 ($p=0.059$)[d]
700		benign	5/17 ($p=0.014$)[d]
		malignant	15/17 ($p=1.45\times10^{-8}$)[d]
		benign or malignant	16/17 ($p=1.32\times10^{-9}$)[d]
0	liver/ kidney	all types	7/20
100			10/21
700			17/17 ($p=2.17\times10^{-5}$)[d]

TABLE 5-5 (cont.)

QUALITY OF EVIDENCE

Strengths of Study:	2,4-DNT was administered by a relevant route of exposure at 3 dose levels (spaced at equal logarithmic intervals) to both males and females. The experiment was designed to last the entire lifespan of the animals. Gross examinations and histopathology analyses were extensive.
Weakness of Study:	Analysis of the test compound detected $\sim$2% 2,6-DNT, which may affect tumor formation. Few animals survived the intended length of the study (control group survival rate was only 20-30%).
Overall Adequacy:	Adequate
Comments:	Mice that died before 12 months were excluded from this table. Because a majority of the high-dose (5000 ppm) mice died before 12 months, this entire group was excluded.

---

[a] Source: Ellis et al., 1979

[b] Benign = cystic papillary adenomas; malignant = solid renal cell carcinomas, cystic papillary carcinomas.

[c] Many animals died before 24 months. They were nevertheless included in the tumor incidence data.

[d] Fischer's Exact test performed at SRC.

In an extensive study conducted by CIIT (1982), groups of 130 male and 130 female Fischer 344 were administered technical grade DNT at dietary levels that provided 0, 3.5, 14.0 and 35.0 mg/kg/day. The DNT mixture consisted of 76.4% 2,4-, 18.8% 2,6-, 2.4% 3,4-, 1.5% 2,3- and <1.0% 2,5- and 3,5-DNT. Ten rats of each sex/group at weeks 26 and 52, 20 rats of each sex/group at week 78, all surviving high-dose rats at week 55 and those surviving at termination were sacrificed and necropsied. All rats either found dead or sacrificed in a moribund condition were also necropsied. Extensive histopathological examinations were performed on tissues from these rats. Throughout the course of the study, 10 control males, 12 control females, 14 low-dose males, 15 low-dose females, 48 middle-dose males, 9 middle-dose females, 8 high-dose males and 2 high-dose females died. In addition, 9 control males, 11 control females, 6 low-dose males, 14 low-dose females, 19 middle-dose males, 13 middle-dose females and 1 high-dose male were sacrificed in extremis.

After 26 weeks, two high-dose males had hepatocellular carcinomas that were considered to be induced by technical grade DNT. Of those animals found dead or sacrificed in extremis through week 52, three high-dose males and one high-dose female had hepatic nodules. There was a dose-related increase in the incidence of hepatocellular carcinomas in high- and middle-dose males sacrificed after 52 weeks, while high-dose females also had a significant increase in the frequency of this lesion. Cholangiocarcinomas, an unusual neoplasm in this strain of rat that presumably is derived from bile duct epithelium, were also observed in two high-dose males and were considered treatment-related.

All high-dose rats were terminated at week 55 because of a significantly decreased survival rate. Only 20 rats of each sex were examined histologically. Hepatocellular carcinomas were present in all males and 11 females, while neoplastic nodules were observed in 5 males and 12 females. Cholangiocarcinomas were found in three males, while two cases of hepatocellular-cholangiocarcinoma were reported in males.

Dietary intake of technical grade DNT through 78 weeks resulted in a high incidence of hepatocellular carcinomas in middle-dose males and a significant incidence of hepatic neoplastic nodules in middle-dose males and females. Cholangiocarcinomas also appeared in middle-dose males to a notable extent. Some fairly common benign lesions including mammary fibroadenomas and subcutaneous fibromas in middle-dose males and pituitary chromophobe adenomas in middle-dose females were also observed although their significance was somewhat equivocal.

Similar, but more frequent, lesions occurred following 104 weeks of treatment. The incidences of liver hepatocellular carcinomas, neoplastic nodules and hepatocellular carcinoma or neoplastic nodule are given in Table 5-6. There was a dose-related increase in the incidence of hepatocellular carcinomas in males; seven of the middle-dose males had pulmonary metastasis. Of the 40 middle-dose females having hepatocellular carcinomas, one had pulmonary metastasis. The incidence of hepatic neoplastic nodules also appeared to be dose-related in both males and females. As with animals observed at 78 weeks, there were dose-related increases in a number of relatively common benign neoplasms; mammary fibroadenomas and subcutaneous fibromas in both sexes and testicular mesotheliomas in males. A similar pattern of lesions was observed in those animals found dead or sacrificed *in extremis* throughout the study (see Table 5-6).

TABLE 5-6

Incidence of Liver Tumors in Fischer 344 Rats Treated with Technical-Grade DNT in the Diet[a,b]

Sex	Dose (mg/kg/day)	Duration of Treatment[c] (weeks)	Tumor Type	Tumor Incidence[d] (p value)
M	0	52	hepatocellular carcinoma or neoplastic nodule	0/10
	3.5	52	neoplastic nodule	0/10
	14	52	hepatocellular carcinoma neoplastic nodule hepatocellular carcinoma or neoplastic nodule	3/10 (NS) 4/10 (0.043) 5/10 (0.016)
	35	52	hepatocellular carcinoma neoplastic nodule hepatocellular carcinoma or neoplastic nodule	10/10 ($5x10^{-6}$) 3/10 (NS) 10/10 ($5x10^{-6}$)
F	0	52	hepatocellular carcinoma or neoplastic nodule	0/10
	3.5	52	hepatocellular carcinoma or neoplastic nodule	0/10
	14	52	hepatocellular carcinoma or neoplastic nodule	0/10
	35	52	hepatocellular carcinoma neoplastic nodule hepatocellular carcinoma or neoplastic nodule	4/10 (0.043) 8/10 ($3.6x10^{-4}$) 9/10 ($6x10^{-5}$)
M	35	55[e]	hepatocellular carcinoma neoplastic nodule hepatocellular carcinoma or neoplastic nodule	20/20 ($3.3x10^{-6}$)[f] 5/20 (NS)[f] 20/20 ($3.3x10^{-6}$)[f]
F	35	55[e]	hepatocellular carcinoma neoplastic nodule hepatocellular carcinoma or neoplastic nodule	11/20 ($3.1x10^{-6}$)[f] 12/20 ($1.5x10^{-5}$)[f] 19/20 ($3.7x10^{-6}$)[f]
M/F	0	78	hepatocellular carcinoma or neoplastic nodule	0/20
M	3.5	78	neoplastic nodule	1/20 (NS)
	14	78	hepatocellular carcinoma neoplastic nodule hepatocellular carcinoma or neoplastic nodule	19/20 ($1.5x10^{-10}$) 11/20 ($7.3x10^{-5}$) 20/20 ($7.3x10^{-12}$)
F	3.5	78	neoplastic nodule	2/20 (NS)
	14	78	neoplastic nodule	10/20 ($2.8x10^{-4}$)

TABLE 5-6 (cont.)

Sex	Dose (mg/kg/day)	Duration of Treatment[c] (weeks)	Tumor Type	Tumor Incidence[d] (p value)
M	0	104	hepatocellular carcinoma	1/61
			neoplastic nodule	9/61
			hepatocellular carcinoma or neoplastic nodule	10/61
M	3.5	104	hepatocellular carcinoma	9/70 (0.015)
			neoplastic nodule	11/70 (NS)
			hepatocellular carcinoma or neoplastic nodule	19/70 (NS)
	14	104	hepatocellular carcinoma	22/23 (5.6×10^{-18})
			neoplastic nodule	16/23 (3×10^{-8})
			hepatocellular carcinoma or neoplastic nodule	23/23 (3.7×10^{-19})
F	0	104	hepatocellular carcinoma	0/57
			hepatocellular carcinoma or neoplastic nodule	5/57
	3.5	104	neoplastic nodule	12/61 (NS)
	14	104	hepatocellular carcinoma	40/68 (1.2×10^{-14})
			neoplastic nodule	53/68 (8.7×10^{-16})
			hepatocellular carcinoma or neoplastic nodule	66/68 (10×10^{-27})
M	0	NR[g]	hepatocellular carcinoma or neoplastic nodule	0/19
	3.5	NR[g]	hepatocellular carcinoma	1/20 (NS)
			neoplastic nodule	3/20 (NS)
			hepatocellular carcinoma or neoplastic nodule	4/20 (NS)
M	14	NR[g]	hepatocellular carcinoma	57/65 (6.7×10^{-18})
			neoplastic nodule	31/65 (3.6×10^{-5})
			hepatocellular carcinoma or neoplastic nodule	60/65 (1.3×10^{-14})
F	0	NR[g]	hepatocellular carcinoma	0/23
			neoplastic nodule	1/23
			hepatocellular carcinoma or neoplastic nodule	1/23
	3.5	NR[g]	hepatocellular carcinoma	1/29 (NS)
			neoplastic nodule	1/29 (NS)
			hepatocellular carcinoma or neoplastic nodule	2/29 (NS)
	14	NR[g]	hepatocellular carcinoma	1/22 (NS)
			neoplastic nodule	12/22 (2.3×10^{-5})
			hepatocellular carcinoma or neoplastic nodule	13/22 (7×10^{-6})

TABLE 5-6 (cont.)

QUALITY OF EVIDENCE

Strengths of Study:	Technical grade DNT was administered by a relevant route at three dose levels to both sexes of rats. A lifetime exposure (104 weeks) was used. Sufficient numbers of animals were used and the survival rate was high enough to permit an assessment of the risk of lifetime exposure. Histological examinations were comprehensive.
Weakness of Study:	Only one species was studied. There is no way to determine which isomer(s) is primarily responsible for the observed effects.
Overall Adequacy:	Adequate

[a] Source: CIIT, 1982

[b] 76.4% 2,4-, 18.8% 2,6-, 2.4% 3,4-, 1.5% 2,3-, and <1.0% 2,5-DNT

[c] Duration of study equals duration of treatment

[d] Fisher Exact Test performed at SRC

[e] All high-dose animals were sacrificed at 55 weeks. Twenty rats were examined histologically. No other dose group was analyzed at this time point.

[f] Compared with controls of rats killed at 52 weeks

[g] These results are from animals found dead or sacrificed in extremis throughout the entire study.

NR = Not reported; NS = not significant

A comparison of these studies indicates that while purified 2,4-DNT (>95%) produced no malignant tumors (NCI, 1978) or a weak though significant hepatocarcinogenic response (Ellis et al., 1979), technical grade DNT containing 76% 2,4-DNT and 19% 2,6-DNT induced malignant lesions in 100% of the animals after 12 months (CIIT, 1982). This would suggest that an isomer other than 2,4-DNT may be of primary importance in the carcinogenic response observed in the CIIT study (Popp and Leonard, 1982). This led investigators to test the *in vivo* initiating and promoting abilities of all DNT isomers separately (Popp and Leonard, 1982; Leonard et al., 1983; Slaga et al., 1985).

Oral administration of the six individual DNT isomers as well as technical grade DNT to male CDF(F-344)/Cr/Br rats at a dose of 75 mg/kg followed by a partial hepatectomy 12 hours later resulted in weak initiating activity in the liver as evaluated by the presence of GGT+ foci only by technical grade DNT and purified 2,6-DNT (Popp and Leonard, 1982; Leonard et al., 1983). In another experiment, both 2,4- and 2,6-DNT were tested to investigate possible hepatic promoting activity (Popp and Leonard, 1982). Rats initiated with a single intraperitoneal injection of 150 mg/kg diethylnitrosamine were fed 2,4- or 2,6-DNT in the diet for 12 weeks at dose levels of 27 or 7 mg/kg/day, respectively. These doses approximated the amount of the individual isomers ingested by rats receiving 35 mg/kg/day of technical grade DNT in the CIIT (1982) bioassay. The number of GGT+ foci was significantly enhanced by both isomers, although 2,6-DNT produced a more dramatic response. These results suggest that only 2,6-DNT has detectable initiating activity, while the 2,4- and 2,6-isomers possess hepatic promoting activity (Popp and Leonard, 1982). Therefore, the induction of the neoplastic response by technical grade DNT was presumably due to the initiating ability of 2,6-DNT while the development of neoplasms relied on the promoting activity of both the 2,4- and 2,6-isomers.

Analysis of these studies suggests that 2,6-DNT is a complete carcinogen, which could produce tumors when administered alone. Popp and Leonard (1982) reported that 99.9% purified 2,6-DNT fed to an unspecified number of animals (the species was not specified but is assumed to be rats) at a daily dose of 14 mg/kg/day induced a 100% incidence of hepatocellular carcinomas after 1 year, while at a daily dose of 7 mg/kg/day, a 95% incidence was observed. Alternatively, 2,4-DNT fed for 1 year at a level of 27 mg/kg/day produced no hepatocellular carcinomas. Thus, 2,6-DNT appears to be primarily responsible for the hepatocarcinogenic activity of technical grade DNT reported in the CIIT (1982) study.

Support for the initiating and promoting abilities of 2,4- and 2,6-DNT was provided by Slaga et al. (1985) in a study that examined the ability of DNT isomers to induce skin tumors in SENCAR mice and lung tumors in Strain A mice. When applied topically at doses of 1, 5 or 10 mg for 2,4- and 2,6-DNT and 2, 10 or 50 mg for the other DNT isomers followed by weekly applications of 4 μg TPA for 30 weeks, only 2,6-DNT produced a higher percentage of mice with squamous cell carcinomas (5%), although this result was not statistically significant. When given intraperitoneally at a dose of 10 mg/kg followed by weekly TPA applications, 2,6-DNT produced a 10% incidence of mice with carcinoma but this was also not statistically significant. Histological studies indicated that both 2,4- and 2,6-DNT were very effective in inducing inflammation and hyperplasia when applied to the skin, suggesting that they may have skin tumor promoting ability (Slaga et al., 1985).

In the lung tumor study (Slaga et al., 1985), A/Jax male mice were injected intraperitoneally with three doses ranging up to 6000 mg/kg for 3,4- and 3,5-DNT, 4800 mg/kg for 2,3-, 2,5- and 2,6-DNT and 1200 mg/kg for 2,4-DNT 3 times/week for 8 weeks and sacrificed 16 weeks after the last injection. Although there were indications of a dose response relationship for 2,3-, 2,4- and 2,6-DNT, none of these compounds produced a tumor incidence significantly above that of the vehicle control groups.

Similar results were obtained by Stoner et al. (1984) who also used the Strain A mouse lung tumor bioassay to test 2,4-DNT, 2,6-DNT and a mixture of the two isomers for potential carcinogenic effects. Twenty six male and female A/Jax mice were administered 2,6-DNT, 2,4-DNT and a 2:1 mixture of 2,4- and 2,6-DNT either orally or by intraperitoneal injection. The dose levels were derived from preliminary experiments, which determined the MTD for each compound or mixture. Each compound was tested at three dose levels: the MTD, 0.5 MTD and 0.2 MTD. The doses used were as follows: 600, 1500 and 3000 mg/kg for 2,4-DNT and 2,6-DNT (intraperitoneal); 960, 2400 and 4800 mg/kg for 2,4-DNT 2,6-DNT (2:1) (intraperitoneal); and 1200, 3000 and 6000 mg/kg for all orally administered compounds. The doses were given 3 times/week for 8 weeks (intraperitoneal) or 2 times/week for 12 weeks (oral). Appropriate vehicle controls and positive controls treated with 3-methycholanthrene or urethan were used. Mice were sacrificed after 30 weeks and the lungs were removed, fixed and examined for adenomas. The survival rate after 30 weeks was high, averaging 85-90% and never falling below 75% for any dose group. Despite the high dose levels used in this study, 2,4-DNT, 2,6-DNT and the 2:1 mixture of 2,4- and 2,6-DNT at any dose level by either exposure did not increase the incidence of lung tumors compared with controls. The positive controls responded appropriately.

5.2. MUTAGENICITY

Table 5-7 summarizes the numerous studies regarding DNT genotoxicity. Hodgson et al. (1976), using Salmonella typhimurium TA1535, showed that 2,4-DNT was capable of producing base-pair mutations, while Chiu et al. (1978) demonstrated that this isomer was unable to induce mutations in TA98 and TA100. A comprehensive analysis of the mutagenic potential of individual DNT isomers and technical grade DNT demonstrated that all compounds were weakly mutagenic in at least one strain of S. typhimurium in the Salmonella/microsome test (Couch et al., 1981). The most potent isomer was 3,5-DNT which increased the number of revertants to histidine prototrophy in TA98 and TA1538 by 50- and 40-fold, respectively, when compared with controls, indicating that this isomer is a frameshift-inducing mutagen. All other isomers, except 3,4-DNT, produced a response in these strains, although to a lesser degree. In addition, all compounds, except technical grade DNT, produced significant reversions in TA100 and/or TA1535, which respond to agents inducing base-pair substitutions or frameshift mutations. TA1537 was the least responsive, having a maximal reversion rate 7 times greater than controls in the presence of 2,3-DNT. In general, responses were concentration-dependent and the addition of rat-liver S-9 often reduced the number of revertants per plate. A quantitative bacterial suspension assay using strain TA98, which was the most responsive to the mutagenic effects of DNTs, produced a concentration-related response for all isomers with 3,5-DNT again being the most mutagenic (Couch et al., 1981). 2,4- and 2,6-DNT were only able to induce a 5-fold increase in the mutation rate of TA98 at concentrations of 500 and 1000 μg/mℓ, respectively. The addition of S-9 generally resulted in decreased mutagenicity and decreased cytotoxicity. This study also used a forward mutation assay in S. typhimurium TM677 using

TABLE 5-7

Mutagenicity Testing of DNTs

Assay	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA1535	2,4-DNT/ NR	plate incorporation	NR	NR	+	No details were given	Hodgson et al., 1976
Reverse mutation	S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	2,6-DNT/ NR	plate incorporation	50-1000 µg	none	+ + - + +	NC	Simmon et al., 1977
Reverse mutation	S. typhimurium TA98 TA100	2,4-DNT/ NR	plate incorporation	0.1-10 µmole	none	- -	NC	Chiu et al., 1978
Reverse mutation	S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	2,3-DNT/ NR	plate incorporation	200 µg	±S-9	+ + + + - - - + + +	Highest nontoxic dose tested; S-9 from Aroclor-induced rats	Couch et al., 1981
Reverse mutation	S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	2,4-DNT/ ≥99.98%	plate incorporation	200 µg	±S-9	- + - - - + - + - +	Highest nontoxic dose tested; S-9 from Aroclor-induced rats	Couch et al., 1981
Reverse mutation	S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	2,5-DNT/ NR	plate incorporation	200 µg	±S-9	- + - + + + - - - +	Highest nontoxic dose tested; S-9 from Aroclor-induced rats	Couch et al., 1981
Reverse mutation	S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	2,6-DNT/ ≥99.98%	plate incorporation	1000 µg	±S-9	- + - + + + + - - +	Highest nontoxic dose tested; S-9 from Aroclor-induced rats	Couch et al., 1981

TABLE 5-7 (cont.)

Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	3,4-DNT/ ≥99.98%	plate incorporation	200 µg	±S-9	 - - - - - + - - - -	Highest nontoxic dose tested; S-9 from Aroclor-induced rats	Couch et al., 1981
S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	3,5-DNT/ ≥99.98%	plate incorporation	40 µg	±S-9	 - + + + - - - + + +	Highest nontoxic dose tested; S-9 from Aroclor-induced rats	Couch et al., 1981
S. typhimurium TA98 TA100 TA1535 TA1537 TA1538	tDNT/ >99.9%	plate incorporation	1000 µg	±S-9	 + + - - - - - - + +	Highest nontoxic dose tested; S-9 from Aroclor-induced rats	Couch et al., 1981
S. typhimurium TA98 TA100	2,4-DNT/ NR	plate incorporation	500 µg 1000 µg	±S-9	 - - + +	S-9 from Aroclor 1254-induced rats	Tokiwa et al., 1981
S. typhimurium TA98 TA100	2,6-DNT/ NR	plate incorporation	1000 µg	±S-9	 - - - +	S-9 from Aroclor 1254-induced rats	Tokiwa et al., 1981
S. typhimurium TA98 TA100	3,4-DNT/ NR	plate incorporation	1000 µg	±S-9	 - - - +	S-9 from Aroclor 1254-induced rats	Tokiwa et al., 1981
S. typhimurium TA98 TA100	2,4-DNT/ >99%	plate incorporation	100-300 µg	none	 + +	2,4-DNT considered a weak mutagen since the number of revertants/plate induced by this compound was <3-fold that induced spontaneously	Mori et al., 1982
S. typhimurium TA1535 TA1537 TA1538 TA98 TA100 TA100 NR3	2,3-DNT/ NR	plate incorporation	50-1000 µg	±S-9	 - - - - - + - + + + - -	S-9 from Aroclor-induced rats	Spanggord et al., 1982

TABLE 5-7 (cont.)

ay	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
e n	*S. typhimurium* TA1535 TA1537 TA1538 TA98 TA100 TA100 NR3	2,4-DNT/ NR	plate incorporation	10-5000 μg	±S-9	 - - - - - - - - - + + -	S-9 from Aroclor-induced rats	Spanggord et al., 1982
? on	*S. typhimurium* TA1535 TA1537 TA1538 TA98 TA100 TA100 NR3	2,5-DNT/ NR	plate incorporation	50-300 μg	±S-9	 + + - - + + + + + + - -	S-9 from Aroclor-induced rats	Spanggord et al., 1982
e on	*S. typhimurium* TA1535 TA1537 TA1538 TA98 TA100 TA100 NR3	2,6-DNT/ NR	plate incorporation	10-5000 μg	±S-9	 - - - - - - - - + + - -	S-9 from Aroclor-induced rats	Spanggord et al., 1982
n	*S. typhimurium* TA1535 TA1537 TA1538 TA98 TA100 TA100 NR3	3,4-DNT/ NR	plate incorporation	10-5000 μg	±S-9	 - - - - - - - - + + - -	S-9 from Aroclor-induced rats	Spanggord et al., 1982
e on	*S. typhimurium* TA1535 TA1537 TA1538 TA98 TA100 TA100 NR3	3,5-DNT/ NR	plate incorporation	100-1100 μg	±S-9	 - - + + + + + + + + - -	S-9 from Aroclor-induced rats	Spanggord et al., 1982
pair	male Fischer 344 rat hepatocytes	2,3-DNT/ NR	cell culture	10-100 μM	none	-	NC	Bermudez et al., 1979
pair	male Fischer 344 rat hepatocytes	2,5-DNT/ NR	cell culture	10-100 μM	none	-	NC	Bermudez et al., 1979

TABLE 5-7 (cont.)

ay	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
air	male Fischer 344 rat hepatocytes	3,4-DNT/ NR	cell culture	10-100 µM	none	-	NC	Bermudez et al., 1979
pair	male Fischer 344 rat hepatocytes	3,5-DNT/ NR	cell culture	10-100 µM	none	-	NC	Bermudez et al., 1979
pair	male Fischer 344 rat hepatocytes	2,6-DNT/ NR	cell culture	100-1000 µM	none	-	NC	Bermudez et al., 1979
pair	male Fischer 344 rat hepatocytes	tDNT/NR	cell culture	10-100 µM	none	-	NC	Bermudez et al., 1979
pair	male Fischer 344 rat hepatocytes	2,4-DNT/ NR	cell culture	100-1000 µM	none	-	NC	Bermudez et al., 1979
air	male Fischer 344 rat hepatocytes	tDNT/ >99.9%	gavage	100 mg/kg	NA	+	Repair activity peaked at 12 hour post-treatment	Mirsalis and Butterworth, 1982
pair	female Fischer 344 rat hepatocytes	tDNT/ >99.9%	gavage	100 mg/kg	NA	+	Repair activity much lower compared with males	Mirsalis and Butterworth, 1982
pair	male Fischer 344 rat hepatocytes	tDNT/ >99.9%	in the diet for 4 weeks	0.1%	NA	+	Repair activity observed at weeks 1, 2 and 4	Mirsalis and Butterworth, 1982
pair	male Fischer 344 rat hepatocytes	2,4-DNT/ >98.0%	gavage	100 mg/kg	NA	+	Response was weak compared with tDNT	Mirsalis and Butterworth, 1982
pair	male Fischer 344 rat hepatocytes	2,6-DNT/ >99.9%	gavage	5-20 mg/kg	NA	+	Response at 20 mg/kg comparable with that observed with 100 mg/kg tDNT	Mirsalis and Butterworth, 1982
pair	male Fischer 344 rat hepatocytes	tDNT/NR	gavage	100 mg/kg	NA	+	Response measured 12 hours after dosing	Mirsalis et al., 1982
pair	axenic male Fischer 344 rat hepatocytes	tDNT/NR	gavage	100 mg/kg	NA	-	Response measured 12 hours after dosing	Mirsalis et al., 1982
pair	male Fischer 344 rat spermatocytes	2,4-DNT/ >99.9%	cell culture	10-100 µM	none	-	1000 µM was toxic to the cells _in vitro_	Working and Butterworth, 1984
pair	male Fischer 344 rat spermatocytes	2,6-DNT/ >99.9%	cell culture	10-100 µM	none	-	1000 µM was toxic to the cells _in vitro_	Working and Butterworth, 1984

TABLE 5-7 (cont.)

ay	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
ome ion	rat lymphocyte and kidney cultures	2,4-DNT/ NR	fed in diet for 19 weeks	0.2%	NA	-	No details were given	Hodgson et al., 1976
ome ion	CD rat lymphocyte and kidney cultures	2,4-DNT/ NR	fed in diet for 19 weeks	265 mg/kg/day	NA	+	Increased chromatid breaks and gaps	Lee et al., 1978
ome ion	CD rat lymphocyte and kidney cultures	2,6-DNT/ NR	fed in diet for 13 weeks	37 mg/kg/day	NA	+	Increased number of tetraploid chromosomes (kidney); increased chromatid breaks and gaps (lymphocytes)	Lee et al., 1978
ome ion	beagle dog bone marrow and kidney cultures	2,4-DNT/ 98.0%	fed in diet for 24 weeks	10 mg/kg/day	NA	-	NC	Ellis et al., 1979
ome ion	CD rat bone marrow and kidney cultures	2,4-DNT/ 98.0%	fed in diet for 24 weeks	0.01-0.07%	NA	-	NC	Ellis et al., 1979
ian c cell on	CHO/HGPRT system	tDNT/ 99.9%	cell culture	2.0 mM	±S-9	- -	S-9 from Aroclor-induced rats	Abernethy and Couch, 1982
ian c cell on	CHO/HGPRT system	2,3-DNT/ NR	cell culture	0.1 mM	±S-9	- -	S-9 from Aroclor-induced rats	Abernethy and Couch, 1982
ian c cell on	CHO/HGPRT system	2,4-DNT/ ≥99.98%	cell culture	3.0 mM	±S-9	- -	S-9 from Aroclor-induced rats	Abernethy and Couch, 1982
ian c cell on	CHO/HGPRT system	2,5-DNT/ NR	cell culture	0.5 mM	±S-9	- -	S-9 from Aroclor-induced rats	Abernethy and Couch, 1982
ian c cell on	CHO/HGPRT system	2,6-DNT/ ≥99.98%	cell culture	2.5 mM	±S-9	- -	S-9 from Aroclor-induced rats	Abernethy and Couch, 1982
ian c cell on	CHO/HGPRT system	3,4-DNT/ ≥99.98%	cell culture	0.1 mM	±S-9	- -	S-9 from Aroclor-induced rats	Abernethy and Couch, 1982

TABLE 5-7 (cont.)

y	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
ell	CHO/HGPRT system	3,5-DNT/ ≥99.98%	cell culture	2.0 mM	±S-9	- -	S-9 from Aroclor-induced rats	Abernethy and Couch, 1982
	S. typhimurium TM677	2,3-DNT/ NR	plate incorporation	75 μg/mℓ	±S-9	- -	S-9 from Aroclor-induced rats	Couch et al., 1981
	S. typhimurium TM677	2,4-DNT/ ≥99.98%	plate incorporation	500 μg/mℓ	±S-9	+ +	S-9 from Aroclor-induced rats	Couch et al., 1981
	S. typhimurium TM677	2,5-DNT/ NR	plate incorporation	250 μg/mℓ	±S-9	+ +	S-9 from Aroclor-induced rats	Couch et al., 1981
	S. typhimurium TM677	2,6-DNT/ ≥99.98%	plate incorporation	500 μg/mℓ	±S-9	+ +	S-9 from Aroclor-induced rats	Couch et al., 1981
	S. typhimurium TM677	3,4-DNT/ ≥99.98%	plate incorporation	100 μg/mℓ	±S-9	- +	S-9 from Aroclor-induced rats	Couch et al., 1981
	S. typhimurium TM677	3,5-DNT/ ≥99.98%	plate incorporation	50 μg/mℓ	±S-9	+ +	S-9 from Aroclor-induced rats	Couch et al., 1981
	S. typhimurium TM677	tDNT/ >99.9%	plate incorporation	500 μg/mℓ	±S-9	+ +	S-9 from Aroclor-induced rats	Couch et al., 1981
le reaks	rat hepatocytes	2,4-DNT/ NR	cell culture	0.03-3.0 mM	none	+	Only highest dose (3 mM) gave a significant response	Sina et al., 1983
le reaks	rat hepatocytes	2,3-DNT/ NR	cell culture	0.03-3.0 mM	none	+	Lowest dose (0.03 mM) did not produce a significant response	Sina et al., 1983
le reaks	rat hepatocytes	2,6-DNT/ NR	cell culture	0.03-3.0 mM	none	+	Only highest dose (3 mM) gave a significant response	Sina et al., 1983
le reaks	rat hepatocytes	3,4-DNT/ NR	cell culture	0.03-3.0 mM	none	+	Lowest dose (0.03 mM) did not produce a significant response	Sina et al., 1983
n ation	P388 mouse lymphoma (TK +/-)	2-4-DNT/ NR	cell culture	100-500 mM	±S-9	- +	Source of S-9 not reported	Styles and Cross, 1983
n ation	P388 mouse lymphoma (TK +/-)	2-6-DNT/ NR	cell culture	NR	±S-9	- -	Source of S-9 not reported	Styles and Cross, 1983

TABLE 5-7 (cont.)

	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
ion	P388 mouse lymphoma (TK +/-)	tDNT/ NR	cell culture	NR	±S-9	- -	Source of S-9 not reported	Styles and Cross, 1983
	Sprague-Dawley rats	2,4-DNT/ NR	gavage	60 mg/kg/day 120 mg/kg/day 240 mg/kg/day for 5 days	NA	- - +	NC	Borzelleca and Carchman, 1982
	rats	2,4-DNT/ NR	fed in diet for 13 weeks	0.2%	NA	+	No details were given	Hodgson et al., 1976
	CD rats	2,4-DNT/ 98.0%	fed in diet for 13 weeks	0.02%	NA	-	NC	Ellis et al., 1979
	DBA/2J mice	2,4-DNT/ NR	oral or i.p. injection for 2 consecutive days	250 mg/kg/day	NA	-	NC	Soares and Lock, 1980
	DBA/2J mice	3,5-DNT/ NR	oral or i.p. injection for 2 consecutive days	250 mg/kg/day	NA	-	NC	Soares and Lock, 1980
y	DBA/2J mice	2,4-DNT/ NR	oral or i.p. injection for 2 consecutive days	250 mg/kg/day	NA	-	NC	Soares and Lock, 1980
e	T stock or C57BL/6J mice	tDNT/ >99.9%	i.p. injection	100 mg/kg	NA	-	NC	Soares and Lock, 1980

reported; NC = no comment; tDNT = technical grade DNT; NA = not applicable

8-azaquanine resistance as a genetic marker. Results were similar to those obtained in the reversion assays with all isomers producing concentration-related increases in the mutation rate. Responses were weak compared with known mutagens, and the addition of S-9 to the incubation generally was without effect. In this assay, however, the greater mutagenicity of 3,5-DNT compared with the other isomers was not discernable (Couch et al., 1981).

A similar study demonstrated a modest response in TA100 and no appreciable activity in TA98 with 2,4-, 2,6- and 3,4-DNT (Tokiwa et al., 1981). This report also indicated that both 2,4- and 2,6-DNT were frameshift-type mutagens, producing a significant response in TA1536, TA1537 and TA1538, but not in TA1535. Similarly, Simmon et al. (1977) reported that 2,6-DNT was mutagenic in TA1537, TA1538, TA98 and TA100, but not in TA1535. Spanggord et al. (1982) reported weak activity induced by 2,4- and 2,6-DNT in TA100, the most responsive strain used in the assay. The mutagenic potency, defined as the number of revertants per μg, tested in TA100 without S-9 mix, was greatest for 2,5-DNT (1.29) and 3,5-DNT (1.04), while the potencies for the other isomers ranged between 0.25 and 0.30. No significant activity was detected for any isomer in TA100 NR3, a nitroreductase-deficient strain, indicating that the mutagenic response was dependent on bacterial nitroreductase activity. The authors concluded that the observed mutagenic responses for the different isomers may, in part, depend on the orientation of the nitro groups with a para configuration being more susceptible to nitrosoreduction.

Since 2,4-DNT is, at best, a weak mutagen, metabolites of 2,4-DNT were tested for mutagenicity in TA98 and TA100 to identify the potent mutagen (Mori et al., 1982). While 2,4-diaminotoluene, 2-nitro-4-acetylaminotoluene, 2-amino-4-acetylaminotoluene, 2-amino-4-acetylaminobenzoic acid and

2,4-dinitrobenzoic acid induced no response in either strain, 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2-amino-4-nitrobenzyl alcohol, 4-amino-2-nitrobenzyl alcohol and 2,4-dinitrobenzyl alcohol were weakly mutagenic only at mM concentrations. In contrast, 2,4-dinitrobenzaldehyde, a putative metabolite of 2,4-DNT, produced a strong mutagenic response even at μM concentrations leading the authors to suggest that the formation of this metabolite may be correlated with 2,4-DNT hepatocarcinogenesis (Mori et al., 1982).

DNA repair assays, both _in vitro_ and _in vivo_, have been extensively used as an indicator of DNT mutagenic potential. The significant induction of UDS in primary male Fischer 344 rat hepatocytes was not observed upon the addition of any of the DNT isomers or technical grade DNT to the culture medium (Bermudez et al., 1979). Similarly, the addition of 2,4- or 2,6-DNT to Fischer 344 rat spermatocyte cultures failed to induce DNA repair, as measured by UDS (Working and Butterworth, 1984). In unpublished observations, neither 2,4- or 2,6-DNT were able to elicit activity in an _in vitro_ human hepatocyte DNA repair assay (Rickert et al., 1984).

Unlike plate incorporation assays, the _in vivo-in vitro_ hepatocyte DNA repair test incorporates normal physiological processes such as absorption, metabolism and excretion, which are important in assessing the overall toxicity of the DNTs (see Chapter 4). Male and female rats were administered single doses of DNT isomers by gavage and, at various time points, their livers were perfused and hepatocytes isolated. Net grains/nucleus calculated from quantitative autoradiographic counts reflected the amount of UDS, while a count of the number of hepatocytes in S-phase indicated the extent of cell proliferation (Mirsalis and Butterworth, 1982). A single dose of 100 mg/kg technical grade DNT in male rats resulted in a peak of repair activity 12 hours post-treatment that returned to control levels by 48 hours post-treatment, while a 50-fold increase in the percentage of cells in S-phase was observed 48 hours after dosing. In contrast, a dose of 100 mg 2,4-DNT/kg produced a weak response at 12 hours post-treatment; however, a dose of 2,6-DNT approximately equal to the amount of the isomer present in technical grade DNT (i.e., 20 mg/kg) induced a response comparable to 100 mg/kg technical grade DNT. Rats maintained on a diet containing 0.1% technical grade DNT exhibited small but significant increases in UDS and substantial increases in the number of replicating cells when measured at 1, 2 and 4 weeks. Female rats treated with 100 mg/kg technical grade DNT exhibited a significantly lower level of UDS at 12 hours compared with males, while 48 hours after treatment, males had an almost 8-fold greater increase in the percentage of hepatocytes in the S-phase. These results are consistent with initiation and promotion studies that implicate 2,6-DNT as the isomer primarily responsible for the genotoxic effects of technical grade DNT. In addition, the reduced UDS activity observed in females is in agreement with assays that demonstrate that female rats are less susceptible to the carcinogenic effects of technical grade DNT (see Section 5.1.).

The importance of gut microflora to DNT genotoxicity was demonstrated in axenic and normal male Fischer 344 rats (Mirsalis et al., 1982). While normal rats had a marked increase in UDS 12 hours after a single oral dose of 100/kg technical grade DNT, no repair activity was evident in axenic rats. Thus, reductive metabolism in the gut is necessary for the formation of genotoxic DNT products. This may explain the generally low level of mutagenic activity observed in in vitro assays.

Kidney and bone marrow cells from dogs fed 10 mg 2,4-DNT/kg/day for 24 months had morphologically normal chromosomes. Similar results were observed in rats maintained on a diet containing 0.01 or 0.07% 2,4-DNT (Ellis et al., 1979). Likewise, no increase in the frequency of chromatid breaks or translocations were noted in kidney or lymphocyte cultures from rats fed 0.2% 2,4-DNT for 19 weeks (Hodgson et al., 1976). Lee et al. (1978), however, reported an increased frequency in chromatid breaks and gaps in kidney and lymphocyte cultures derived from CD rats fed up to 265 mg 2,4-DNT/kg/day for 19 weeks. This study also reported an increase in the number of tetraploid chromosomes in kidney cultures and an increased frequency of chromatid breaks and gaps in lymphocytes from CD rats fed up to 37 mg/kg/day 2,6-DNT for 13 weeks.

DNT isomers were tested for mutagenicity and cytotoxicity using the CHO/HGPRT system, an assay designed to measure mammalian genetic cell mutations (Abernethy and Couch, 1982). Although marked differences in cytotoxicity were observed (3,4- and 2,3-DNT were the most toxic), neither technical grade DNT nor any purified DNT isomers induced significant mutagenic activity, with or without added microsomal preparations.

Sina et al. (1983) analyzed DNA single-strand breaks using an alkaline elution/rat hepatocyte assay to predict the mutagenic potential for several DNT isomers. 2,4-DNT induced a 5.1- to 7-fold increase and 2,6-DNT, a >7-fold increase in the elution rate, but only at the highest dose (3 mM), a concentration that resulted in 58 and 85% cell viability for 2,4- and 2,6-DNT, respectively. In contrast, 2,3- and 3,4-DNT at concentrations of 0.3 mM caused DNA damage that was ~7-fold greater than controls. Hepatocyte viability was high at this dose level for 2,3-DNT and 3,4-DNT.

"Pure" 2,4-DNT produced a dose-dependent increase in mutation frequency in the P388 mouse lymphoma gene mutation assay (Styles and Cross, 1983). This increase became insignificant when S-9 mix was added to the incubation medium. 2,6-DNT and technical grade DNT both in the presence and absence of S-9 mix did not induce an increase in mutation.

Dominant lethal assays, which are generally sensitive only to chromosomal anomalies in male germ cells, have been performed by several laboratories and have yielded predominantly negative results. Male CD rats fed 2,4-DNT in the diet at concentrations up to 0.2% for 10 or 13 weeks showed no evidence of dominant lethal mutation effects as the implant viability index was within normal limits (Ellis et al., 1979). Similar results were obtained by Soares and Locke (1980) who treated male DBA/2J mice with oral or intraperitoneal injections of 250 mg/kg 2,4-, 3.5- or technical grade DNT for 2 consecutive days, followed by matings with CD-1 female mice at 48 hours post-treatment. There was no evidence of an increase in dominant lethals as measured by the number of implantations and the percent of postimplantation death. Hodgson et al. (1976) reported that a significant increase in the number of dead implants/total implants was observed in female rats mated with males fed a diet containing 0.2% 2,4-DNT for 13 weeks. No additional details were provided in this abstract. Only slight dominant lethal effects were observed in male Sprague-Dawley rats treated for 5 days with 240 mg/kg/day 2,4-DNT up to 11 weeks after treatment (Borzelleca and Carchman, 1982).

No recessive spots, which are indicative of mutations at specific coat color loci in embryonic neural crest cells in certain strains of mice, were observed in pregnant mice given intraperitoneal injections of 100 mg/kg technical grade DNT (Soares and Locke, 1980). These authors also reported no significant increase in the mean percent of morphologically abnormal sperm in DBA/2J mice 8 weeks after oral or intraperitoneal injections of 250 mg 2,4-DNT/kg/ day for 2 consecutive days.

5.3. TERATOGENICITY

The teratogenic potential of technical grade DNT (76% 2,4-DNT, 19% 2,6-DNT, 2.4% 3,4-DNT, 1.5% 2,3-DNT, <1% 2,5-DNT and <1% 3,5-DNT) was evaluated in timed-pregnant Fischer 344 rats (Price et al., 1985). The rats were administered technical grade DNT in corn oil by gavage on gestation days 7-20 at the following dose levels in mg/kg/day: 14, 35, 37.5, 75, 100 and 150. Twenty-two rats given corn oil only or 200 mg/kg/day hydroxyurea served as vehicle and positive control groups, respectively. Dams were sacrificed and examined on gestation day 20. Maternal mortality was low (<8.0%) for all treatment groups except the 150 mg/kg/day group in which 6/13 animals died before sacrifice. On gestation day 20, dams from the 100 mg/kg/day dose group exhibited signs of DNT toxicity including statistically significant increases in methemoglobin, reticulocyte count, red blood cell size and distribution width, and platelet count as well as significant reductions in red blood cell count and hematocrit. There was a significant dose-related decrease in absolute maternal weight gain (weight gain during treatment minus gravid uterine weight) and significant dose-dependent increases in relative maternal liver and spleen weight.

No differences in the percentage of resorptions, or dead or live fetuses were observed among treatment groups given up to 100 mg/kg/day and the control group, although there was a 49.6% incidence in prenatal mortality in the high-dose group compared with 16.8% in the control group. Parameters relating to live fetuses (number of litters with live fetuses, live litter size, proportion of male fetuses, body weight, crown-rump length, relative liver and spleen weight and placental weight) exhibited no dose-related differences among treatment groups; however, fetuses from the 100 mg/kg/day group had a significantly decreased reticulocyte and red blood cell count and increased red blood cell size. The incidence of gross, visceral and skeletal malformations was low for all DNT-treated groups and did not vary significantly from the control group. In contrast, hydroxyurea produced significant effects on fetal morphological development. Thus, no evidence of a teratogenic effect or a reduction in prenatal viability was exhibited at doses up to 100 mg/kg/day, a dose that produced significant maternal toxicity (Price et al., 1985).

These results are in agreement with Ellis et al. (1979) who observed no significantly increased incidence in gross malformations in CD rats maintained on a diet containing 0.0015, 0.01 or 0.7% 2,4-DNT for 6 months before mating. Selective fetal toxicity was also not observed in timed-mated CD-1 mice administered 390 mg/kg/day 2,4-DNT by gavage on gestation days 7-14 (Smith, 1983). Thus, current data do not support DNT as a teratogenic or fetotoxic agent in mice and rats at doses below which maternal toxicity is expressed.

5.4. OTHER REPRODUCTIVE EFFECTS

Medical and environmental surveys were conducted to evaluate reproductive effects in workers exposed to DNT in a toluene diamine processing plant (Ahrenholz, 1980). Thirty males were divided into three exposure categories. The exposed group (n=9) had the highest current and most likely potential exposure to DNT. The intermediate group (n=12) consisted of those individuals having intermittent exposure over several years but not in the preceding 2 years and the control group (n=9) had no known exposure to DNT during employment at the plant. DNT concentrations measured during the survey ranged from undetectable to 0.10 mg/m^3, calculated as TWAs that are well below the OSHA standard of 1.5 mg/m^3 TWA.

No differences were observed in hepatic and renal blood chemistry profiles between any of the exposure groups. In addition, no statistically significant differences in parameters related to reproductive history were reported, although a small ($p=0.08$) difference did exist between the control and exposed groups regarding the number of spontaneous abortions in the wives of those exposed to DNT. There was also a significant reduction in the number of large morphologic sperm forms in the exposed group. The most notable observation was a statistically significant reduction in the sperm count of workers exposed to DNT compared with the control group, although the sperm count in the nonexposed workers was abnormally high. This report concluded that there was a strong indication that reproductive problems can exist in male workers exposed occupationally to DNT (Ahrenholz, 1980).

In response to the above report, a more comprehensive survey was conducted at another plant site where workers were exposed to DNT and toluene diamine under similar conditions (Hamill et al., 1982). As with the original study, environmental monitoring recorded average DNT levels that were well within acceptable OSHA standards (not otherwise specified). Eighty-four workers exposed to DNT and toluene diamine and 119 controls were subjected to a thorough urogenital examination, a reproductive and fertility questionaire, an estimation of testicular volume, an assessment of serum follicle-stimulating hormone, and an analysis of semen for sperm count and morphology. In contrast to the previous study (Ahrenholz, 1980), no significant alterations in any of these variables were observed in exposed workers. The authors concluded that although toluene diamine and DNT are readily absorbed, no detectable reproductive hazard exists among male workers exposed to these compounds.

Lee et al. (1985) reported decreased spermatogenesis in CD rats fed 0.2% 2,4-DNT in the diet for 13 weeks while a concentration of 0.7% resulted in reduced sperm production by week 4 and aspermatogenesis by week 13. In the same study, groups of 38 male and 38 female rats were maintained on diets containing 0, 0.0015, 0.01 and 0.07% 2,4-DNT. After 12 months, most high-dose males displayed marked atrophy of the testes with severe atrophy of seminiferous tubules accompanied by an almost total absence of spermatogenesis (Table 5-8). Continued treatment resulted in severe testicular degeneration in all dose groups, with the high-dose males having the greatest incidence (see Table 5-8). No female reproductive organ histopathology was observed.

In a related study (Hong et al., 1985), male CD-1 mice exhibited mild degeneration of seminiferous tubules following feeding of 0.7% 2,4-DNT for 13 weeks. Groups of 38 male and 38 female mice fed 0, 0.01, 0.07 or 0.5% 2,4-DNT in the diet for up to 24 months also displayed atrophy of the gonads. In males, decreased spermatogenesis and testicular atrophy were evident in the middle- and high-dose groups (Table 5-9). There was a decrease in testes size as well as a soft consistency to the organ. Microscopically, more differentiated spermatogenic cells were depleted and varying degrees of degeneration in seminiferous tubules were observed. Analogous lesions were present in high-dose females (see Table 5-9), as evidenced by nonfunctioning follicles with a lack of corpora lutea in the ovary, implying an absence of oogenesis.

TABLE 5-8

Incidence of Testicular Atrophy in CD Rats Fed 2,4-DNT*

% in Feed	Intake (mg/kg/day)	Incidence	
		12 Months	>12 Months
0	0	0/7	4/25
0.0015	0.57	0/8	7/27
0.01	3.9	0/8	6/19
0.07	34	6/7	22/27

*Source: Lee et al., 1985

TABLE 5-9

Incidence of Testicular or Ovarian Atrophy in CD-1 Mice Fed 2,4-DNT for 12 or 24 Months*

% in Feed	Intake (mg/kg/day)	Males		Females	
		12 Months	>24 Months	12 Months	>24 Months
0	0	1/8	1/25	0/8	5/23
0.01	14	1/8	17/25	1/8	3/21
0.07	95	1/8	11/20	0/8	5/23
0.5	898	1/8	31/32	5/8	24/25

*Source: Hong et al., 1985

Beagle dogs given 25 mg/kg/day 2,4-DNT orally for 13 weeks had mild to severe testicular degeneration as well as reduced spermatogenesis (Ellis et al., 1985). These effects were not observed in dogs receiving $\geq$10 mg/kg/day. As reviewed by Rickert et al. (1984), dogs receiving up to 100 mg/kg/day 2,6-DNT for 13 weeks and CD rats fed up to 155 mg/kg/day of 2,6-DNT for 13 weeks had testicular atrophy and depressed spermatogenesis (Lee et al., 1976). Severe aspermatogenesis was observed in Swiss mice fed up to 289 mg/kg/day of 2,6-DNT for 4 weeks (Lee et al., 1976). Further details were not reported in the review.

A comprehensive 3-generation reproduction study was performed by Ellis et al. (1979) using CD rats. Parents and offspring were maintained on the same diets containing concentrations of 2,4-DNT that resulted in doses of 0, 0.57, 3.9 or 34 mg/kg/day in males and 0, 0.71, 5.1 or 45 mg/kg/day in females. Both sexes were exposed to the test diets for 6 months before the first mating. Offspring were mated at 3 months of age. Despite the adverse effects of similar doses on germ cell production in males (Lee et al., 1985), no treatment-related effects were observed on fertility, litter size, live-born index, weight at birth, viability index, lactation index, weight at weaning or sex ratio.

These results, combined with those studies involving effects on sperm morphology (Soares and Lock, 1980) and spermatocyte DNA repair (Working and Butterworth, 1984) as well as dominant lethal effects (Borzelleca and Carchman, 1982; Hodgson et al., 1976; Ellis et al., 1979; Soares and Lock, 1980) (see Section 5.2.) indicate that while the DNTs are testicular toxicants, they do not appear to induce genetic damage in male germ cells.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

In an NCI (1978) bioassay, groups of 50 male and 50 female Fischer 344 rats were fed diets containing 0.008 and 0.02% 2,4-DNT for 78 weeks and observed for another 26 weeks. Corresponding control groups containing 25 (low-dose control) and 50 (high-dose control) rats of each sex were observed for 104 weeks. Necropsies were performed on each rat that died, was killed when moribund, or was sacrificed at the end of the bioassay. Gross and microscopic histopathological examinations were conducted on major tissues taken from each rat. The only significant clinical observation was a mean body weight depression that was evident as early as week 16 in high-dose males. At the end of the assay, this group had a mean average body weight ~25% less than controls, while high-dose females exhibited an 18% decrease in mean body weight. The incidence and variety of non-neoplastic, degenerative, proliferative and inflammatory lesions in the major organs were similar in control and treated rats.

A similar protocol was used for B6C3F1 mice (NCI, 1978). Fifty male and female mice were exposed to 2,4-DNT in the diet at concentrations of 0, 0.008 or 0.04% for 78 weeks followed by 13 weeks without treatment. Mean body weight depression was observed in all treated groups by week 30. Approximate weight gain was 91% for low-dose males, 82% for high-dose males, 89% for low-dose females and 76% for high-dose females when calculated as a percentage of the respective control group weight gain. No clinical observations were reported nor was there an increase in the incidence of non-neoplastic, degenerative, proliferative or inflammatory lesions in treated mice compared with controls.

A comprehensive study was conducted by Ellis et al. (1979) to evaluate the subchronic and chronic toxicity of 2,4-DNT in dogs, rats and mice. The study in dogs was also reported by Ellis et al. (1985); the study in rats was also reported by Lee et al. (1985); and the study in mice was also reported by Hong et al. (1985).

For the subchronic study in beagle dogs, groups of four males and four females were given 0, 1, 5 or 25 mg/kg/day of 2,4-DNT in hard gelatin capsules (Ellis et al., 1979, 1985). After 4 weeks and at week 13, one male and one female were sacrificed and the treatment for the remaining dogs was discontinued for 4 weeks, i.e., until week 8 or week 17, to evaluate the reversibility of adverse effects. In the chronic study, groups of six male and six female dogs were fed daily capsules containing 0, 0.2, 1.5 or 10 mg/kg. After 1 year, one pair of dogs was killed for necropsy and another pair was allowed a 4-week recovery period to assess reversibility. A similar procedure was used for the remaining four pairs of dogs at 24 months. A thorough clinical and histopathological examination was performed on each dog following sacrifice.

The primary targets affected by subchronic and chronic oral administration of 2,4-DNT in dogs were the nervous system, erythrocytes and the biliary tract. The major adverse effect was neurotoxicity, characterized by incoordination and paralysis. Effects were observed within 2 months in all dogs receiving 25 mg/kg/day, within 6 months in all dogs given 10 mg/kg/day and during month 16 in one dog given 1.5 mg/kg/day. There was a wide range of variability among individuals as to the onset and severity of the neuropathy. Central nervous system lesions including vacuolation, endothelial proliferation and gliosis of the cerebellum were present in some of the high-dose dogs. The primary erythrocyte effect, methemoglobinemia, was rarely seen in the chronic study, but sequelae, including the presence of Heinz bodies and reticulocytosis, were evident in the middle- and high-dose groups within 3 months. During the second year, these erythrocyte effects were minimal, presumably because of an adaptive response by the dogs. Methemoglobinemia and its sequelae was the only reversible effect in those dogs allowed a 4-week recovery period. Mild biliary hyperplasia was seen in most dogs receiving 10 mg/kg/day and in some dogs given 1.5 mg/kg/day in the chronic study. No other changes were noted in routine clinical and hematological parameters (Ellis et al., 1985).

Groups of 16 male and 16 female CD rats were fed diets containing 0, 0.07, 0.2 or 0.7% 2,4-DNT and 8/sex were sacrificed at the end of 4 and 13 weeks in the rat subchronic assay (Ellis et al., 1979; Lee et al., 1985). A dose-related decrease in feed intake was observed in all treatment groups of both sexes. Weight gain was significantly depressed in both low-dose groups and substantial weight losses occurred in the other treated groups. All high-dose females died during the first 3 weeks, and 6/8 males died between weeks 4 and 13. Abnormal pigmentation of the spleen accompanied by a mild reticulocytosis was evident in the middle- and high-dose groups in both males and females after 4 weeks. Relative kidney, liver and brain weights were increased in male and female middle-dose and male high-dose rats after 13 weeks. In addition, all high-dose rats were anemic. One male from both the middle and high-dose groups had some signs of neuromuscular dysfunction accompanied by demyelination in the cerebellum and brain stem.

In the chronic rat study, diets containing 0, 0.0015, 0.01 and 0.07% 2,4-DNT were administered to groups of 38 males and 38 females (Ellis et al., 1979; Lee et al., 1985). After 12 months, eight males and eight females from each group were killed for necropsy, while the remaining surviving rats were sacrificed after 24 months. Weight gain in high-dose animals was markedly reduced, reaching a plateau after 2 months in females and 4 months in males. Growth in middle-dose males and females began to decrease during month 9, while low-dose rats had growth rates comparable with controls. Anemia, partially compensated as evidenced by increased reticulocyte counts, was prominent in middle- and high-dose males and high-dose females after 12 months. Cumulative deaths for high-dose males and females were significantly higher than controls; 50% mortality occurred in high-dose rats by about month 20 and in controls by about month 23. The most striking lesion was the progressive development of hyperplastic foci in the liver, the severity of which was dose-dependent. These alterations were observed in all male treatment groups and in high-dose females. Neoplastic nodules were also present in the majority of high-dose males and females (see Section 5.1.). Hemosiderosis of the spleen occurred in a majority of high-dose rats.

The subchronic and chronic toxicity of 2,4-DNT was studied in groups of 38 male and 38 female CD-1 mice (Ellis et al., 1979; Hong et al., 1985) using procedures similar to those described for rats by Lee et al. (1985). For the subchronic study, dose levels of 0, 0.07, 0.2 or 0.7% were used. Depressed weight gain was observed in low- and middle-dose males, and males and females fed the highest dose had a slight reduction in feed intake and lost weight. In addition, the high-dose group had mild anemia with reticulocytosis, moderate degeneration of seminiferous tubules, mild hepatocellular dysplasia (also present in some middle-dose males) and pigmentation in Kupffer cells of the liver.

Since the subchronic results indicated less susceptibility to 2,4-DNT in mice compared with rats, dietary levels of 0, 0.01, 0.07 and 0.5% were chosen for mice in the chronic study. Unscheduled deaths occurred considerably sooner in the high-dose mice; all males and females in this group died by month 18 or 21, respectively. Effects on weight gain were more pronounced in males than females. Low- and middle-dose males consistently weighed less than controls after month 11 and 3, respectively, while females in these groups weighed about the same as controls. Weight gain was drastically reduced in all high-dose mice. Toxic anemia and concurrent reticulocytosis occurred in high-dose males and females after 12 months. In addition, all high-dose mice displayed increases in relative spleen and liver weight after 12 months of feeding. A striking finding was an increased incidence of an unidentified, brownish pigment in many tissues of mice at all dietary levels; the intensity was dose-dependent. This pigment was most prominent in the liver and spleen, but was also present in the lung, kidney, adrenals, bone marrow, heart, gonads and central nervous system. Hepatocellular dysplasia, characterized by metabolic, degenerative and hyperplastic alterations of cells was observed in all treated males and high-dose females. Renal tumors in males and testicular or ovarian atrophy were also prominent lesions (see Sections 5.1. and 5.4.).

Kozuka et al. (1979) maintained 20 male Wistar rats on a diet containing 0.5% 2,4-DNT for 6 months. A control group contained 23 rats. The body weights of the treated rats remained relatively stable for the first 6 weeks of treatment and then steadily declined until the end of the study. Mortality was increased in the treated animals, especially during the latter 3 months. An increase in relative organ weight was observed in the liver, spleen and kidney of the exposed rats, while relative testis weight was significantly reduced. A >6-fold increase in the formation of methemoglobin was reported in the treated group after 6 months. After 1 month of treatment, significant increases in a number of serum components (triglycerides, glucose, albumin, albumin/globulin ratio) and serum enzymes (SGOT, SGPT, alkaline phosphase, acid phosphatase) were noted.

The only information pertinent to 2,6-DNT is a 90-day study reported in an abstract (Ellis et al., 1976). This study was essentially the same as that described above for 2,4-DNT (Ellis et al., 1979). Dogs were given oral doses of 2,6-DNT equivalent to 4, 20 or 100 mg/kg/day, while rats and mice were maintained on diets containing 0.01, 0.05 and 0.25% of 2,6-DNT. Both the 2,4- and 2,6-isomers produced similar effects. These included decreased muscular coordination in dogs and rats and decreased appetite and weight loss in all three species. Mortality was increased by the high dose in all animals while the low dose caused no excess mortality. Methemoglobinemia and anemia with reticulocytosis were present in all species. In addition, 2,6-DNT produced several tissue lesions including extramedullary hematopoiesis in the spleen and liver, gliosis and demyelination in the brain, and testicular atrophy with aspermatogenesis. This abstract did not discuss dose-dependent effects nor did it reveal the presence, if any, of sex differences in toxicity.

A rigorous assessment of the carcinogenic and toxicologic effects of technical grade DNT in Fischer 344 rats following dietary administration of 0, 3.5, 14.0 and 35.0 mg/kg/day (130 rats/sex/group) was conducted by CIIT (1982). Ten rats of each sex in each treatment group at weeks 26 and 52, 20 rats/sex/group at week 78, all surviving high-dose rats at week 55, and all surviving animals at termination were sacrificed and necropsied. There was a statistically significant dose-related decrease in body weight gain in all treated groups throughout the first year of the study. After 54 weeks, a dose-related decrease in mean body weights was observed at all dose levels in both sexes. Analysis of hematological data suggested a low grade regenerative anemia in high-dose males and females. Anemia at this dose level was also indicated by the increased hemosiderin and extramedullary hematopoiesis in the spleens of these rats, which is indicative of enhanced red cell turnover.

Analysis of organ weight data revealed alterations in several organs. Consistent significant increases were observed in relative liver and brain weights in all treated rats, relative kidney weights in all but low-dose males and relative ovarian weights in low- and middle-dose females. Increases and decreases were reported in relative weights in the lung, heart and testes depending on the time point examined. Gross necropsy findings revealed alterations in the liver characterized by white or yellow foci in all treated males and females as well as nodules and tissue masses in middle-dose males and females.

Histopathological findings at 26 weeks revealed an enhancement of spontaneous cardiomyopathy in high-dose males and proliferative and degenerative alterations of the hepatocytes and bile duct epithelium in all middle- and high-dose animals. Dietary intake of technical grade DNT for 52 weeks was associated with hepatotoxicity in all middle- and high-dose animals and low-dose males and was characterized by areas of cell alteration, hyperbasophilia and megalocytosis of hepatocytes and vacuolation and necrosis of individual hepatocytes. Other treatment-related lesions in the high-dose males were observed in the kidney, pancreas, testes, spleen and bone marrow. Following 78 weeks of treatment, primary histologic findings pertained to the increased incidence of neoplasms in the liver. Hepatotoxicity was observed in middle-dose males as was an exacerbation of chronic interstitial nephritis in middle-dose males and females. At the end of 2 years, low- and middle-dose animals had a high incidence of hepatotoxicity as well as chronic interstitial nephritis accompanied by parathyroid hyperplasia and adenoma. In addition, an enhancement of degenerative changes in the adrenal glands was noted in middle-dose rats. In general, treatment-related histologic tissue alterations were characterized by the presence of hepatic neoplasms in all treatment groups, an increased incidence of mammary fibroadenomas in all middle-dose rats and an increase in the incidence of subcutaneous fibromas and sarcomas primarily in middle-dose males (see Section 5.1.).

5.6. OTHER RELEVANT INFORMATION

The acute effects of DNT isomers in several species are summarized in Table 5-10. Characteristic signs of acute DNT intoxication include central nervous system depression, respiratory depression and ataxia. Although there are discrepancies between laboratories, the study by Ellis et al. (1980) suggests that 3,5-DNT consistently displayed the greatest toxicity in mice and rats of both sexes when compared with the other isomers. Individual isomers appeared to be somewhat less toxic in mice than in rats. Of the species studied, cats apparently are the most susceptible to the lethal effects of 2,4-DNT, with an oral LD_{Lo} of 27 mg/kg (Spector, 1956).

Ellis et al. (1980) evaluated the local eye and skin irritation effects of DNT using a modified Draize method in rabbits. 2,3-, 2,5- and 3,4-DNT were mildly irritating to the skin while 2,4-, 2,6- and 3,5-DNT were non-irritating. No isomer produced eye irritation. Dermal sensitivity studies in guinea pigs indicated that 2,6-DNT was mildly allergenic while the other five isomers had no sensitizing activity (Ellis et al., 1980).

Several reports from the older literature suggest that diets high in fat or protein may partially block the reduction in body weight gain and lethality induced by 2,4-DNT in rats (Shils and Goldwater, 1953; Clayton and Baumann, 1944, 1948). Further investigation of these effects has not been reported.

TABLE 5-10

Acute Toxicity of DNTs

Route	Species	Dose (mg/kg)	Effect	Reference
		2,4-DNT		
Oral	rat	50	no deaths	NIOSH, 1977
Oral	rat	268 (180-400)*	LD_{50}	MacEwen and Vernot, 1972
Oral	rat (male)	568 (434-705)*	LD_{50}	Ellis et al., 1980
Oral	rat (female)	650 (520-743)*	LD_{50}	Ellis et al., 1980
Oral	mouse	1625 (1180-2240)*	LD_{50}	MacEwen and Vernot, 1972
Oral	mouse (male)	1954 (1848-2178)*	LD_{50}	Ellis et al., 1980
Oral	mouse (female)	1340 (1205-1500)*	LD_{50}	Ellis et al., 1980
Oral	mouse	500	no deaths in 24 hours	Soares and Lock, 1980
Oral	cat	27	minimum lethal dose	Spector, 1956
Inhalation	rat	200 ppm	no deaths when inhaled for 1 hour	NIOSH, 1977
Intraperitoneal	rat	500	LD_{50} at 24 hours	Soares and Lock, 1980
Subcutaneous	rabbit	200	no deaths, not corrosive to skin	NIOSH, 1977

TABLE 5-10 (cont.)

Route	Species	Dose (mg/kg)	Effect	Reference
		2,6-DNT		
Oral	rat	177 (130-240)*	LD_{50}	Vernot et al., 1977
Oral	rat (male)	535 (397-646)*	LD_{50}	Ellis et al., 1980
Oral	rat (female)	795 (744-944)*	LD_{50}	Ellis et al., 1980
Oral	mouse	1000 (590-1700)*	LD_{50}	Vernot et al., 1977
Oral	mouse (male)	621 (488-721)*	LD_{50}	Ellis et al., 1980
Oral	mouse (female)	807 (725-893)*	LD_{50}	Ellis et al., 1980
		2,3-DNT		
Oral	rat	1122	LD_{50}	Back et al., n.d.
Oral	rat (male)	1102	LD_{50}	Ellis et al., 1980
Oral	rat (female)	911	LD_{50}	Ellis et al., 1980
Oral	mouse	1072	LD_{50}	Back et al., n.d.
Oral	mouse (male)	1372	LD_{50}	Ellis et al., 1980
Oral	mouse (female)	1089	LD_{50}	Ellis et al., 1980

TABLE 5-10 (cont.)

Route	Species	Dose (mg/kg)	Effect	Reference
		2,5-DNT		
Oral	rat	707	LD_{50}	Back et al., n.d.
Oral	rat (male)	616	LD_{50}	Ellis et al., 1980
Oral	rat (female)	650	LD_{50}	Ellis et al., 1980
Oral	mouse	1231	LD_{50}	Back et al., n.d.
Oral	mouse (male)	652	LD_{50}	Ellis et al., 1980
Oral	mouse (female)	659	LD_{50}	Ellis et al., 1980
		3,4-DNT		
Oral	rat	1072	LD_{50}	Back et al., n.d.
Oral	rat (male)	907	LD_{50}	Ellis et al., 1980
Oral	rat (female)	807	LD_{50}	Ellis et al., 1980
Oral	mouse	1414	LD_{50}	Back et al., n.d.
Oral	mouse (male)	859	LD_{50}	Ellis et al., 1980
Oral	mouse (female)	747	LD_{50}	Ellis et al., 1980

TABLE 5-10 (cont.)

Route	Species	Dose (mg/kg)	Effect	Reference
		3,5-DNT		
Oral	rat (male)	309	LD_{50}	Ellis et al., 1980
Oral	rat (female)	216	LD_{50}	Ellis et al., 1980
Oral	mouse (male)	611	LD_{50}	Ellis et al., 1980
Oral	mouse (female)	607	LD_{50}	Ellis et al., 1980
		Technical Grade DNT		
Oral	rat	268	LD_{50} at 48 hours	Soares and Lock, 1980

*95% confidence interval

The initial acute toxic effects resulting from inhalation, ingestion or dermal absorption of 2,4-DNT in humans most probably is methemoglobinemia followed by cyanosis (U.S. EPA, 1980a). Symptoms of exposure typically do not appear for 2-3 hours, unless alcohol has been recently consumed, in which case symptoms of intoxication may appear within 10 or 15 minutes (TSCA-ITC, 1981). Headache is frequently the initial symptom and its intensity increases as the severity of methemoglobinemia progresses. Several other symptoms, including vertigo, fatigue, dizziness, weakness, nausea, vomiting, dyspnea, drowsiness, arthralgia, insomnia, tremor, paralysis, unconsciousness, chest pain, shortness of breath, palpitation, anorexia and weight loss have been reported after exposure to varying doses of 2,4-DNT (U.S. EPA, 1980a).

Reports of human exposure to DNTs, primarily 2,4-DNT, are rare and can be found predominantly in the older literature. Acute effects of 2,4-DNT poisoning including dyspnea, dizziness, sleepiness and pain in the joints have resulted from inhaled vapors and absorption through the skin during the purification of crude 2,4-DNT cakes (Perkins, 1919). Exposure to 2,4-DNT may also result in temporary visual disturbances (Lewin, 1921). Floret (1929) reported a severe case of 2,4-DNT intoxication in which a plant worker suffered from marked cyanosis and complained of headache, heart palpitations, tightness in the chest, insomnia and a lack of appetite. A medical examination of this patient revealed impaired reflexes, nystagmus and tremors of varying intensity in the hands, arms, head, extended fingers and tongue.

An extensive epidemiological study conducted by McGee et al. (1942) examined the health effects in a large group of workers at a plant where 2,4-DNT was used in the manufacture of explosives. A group of 154 workers complained of a variety of symptoms including an unpleasant metallic taste in the mouth, muscular weakness, headache, loss of appetite and dizziness (Table 5-11). Primary clinical findings included pallor, cyanosis and anemia (Table 5-12). In some cases of anemia, the hemoglobin and erythrocyte counts slowly returned to normal. No instances of permanent physical impairment were found. A follow-up study (McGee et al., 1947) consisted of a group of 714 workers, which was considered a lower exposure group because of improvements in plant operation and the skills of the workers. The frequency of clinical signs (cyanosis and anemia) and symptoms (headache) presented by these workers was significantly decreased compared with the earlier, higher exposed group.

5.7. SUMMARY

Several bioassays have been performed regarding the carcinogenicity of DNT and individual DNT isomers. Rats exposed to 80 and 200 ppm 2,4-DNT in the diet for 78 weeks were reported to have a significant dose-related increased incidence of subcutaneous fibromas in males and statistically significant increased frequency of mammary gland fibroadenomas in high-dose females compared with controls (see Table 5-1). Male and female mice exposed to 80 and 400 ppm in the diet for 78 weeks displayed no evidence of a significant tumor incidence in any tissue relative to control groups (NCI, 1978).

Administration of 2,4-DNT to rats at dietary concentrations of 0, 15, 100 and 700 ppm for 2 years resulted in an increase in the number of high-dose animals with hepatocellular carcinomas that was statistically significant only in females (Ellis et al., 1979). High-dose females also had a 97% incidence of benign mammary gland neoplasms (see Table 5-4). This study also reported a statistically significant increase in the number of

TABLE 5-11

Symptoms Exhibited by 154 2,4-DNT-Exposed Workers*

Symptom	Number	Percent
Unpleasant taste in mouth	96	62
Weakness	78	51
Headache	76	49
Inappetence	72	47
Dizziness	68	44
Nausea	57	37
Insomnia	57	37
Pain in extremities	40	26
Vomiting	29	19
Loss of weight (5 lbs or more)	10	6.5
Diarrhea	8	5.2

*Source: McGee et al., 1942

TABLE 5-12

Clinical Signs in 154 2,4-DNT-Exposed Workers*

Symptom	Number	Percent
Pallor	55	36
Cyanosis	52	34
Anemia	36	23
Leukocytosis	19	12
Hypotension	9	5.8
Dermatitis	6	3.9
Leukopenia	5	3.2
Acute toxic hepatitis/jaundice	2	1.4

*Source: McGee et al., 1942

high-dose male mice with benign (cystic papillary adenomas) and malignant (solid renal cell carcinomas, cystic papillary carcinomas) renal tumors following dietary administration of 0, 100 or 700 ppm 2,4-DNT. Hepatomas were also observed in these mice but the incidence was not dose-related (see Table 5-5). Female mice maintained on the same diet had no treatment-related increase in tumor frequency in any tissue.

A 100% incidence of hepatocellular carcinoma was reported in male rats exposed to 35 mg/kg/day technical grade DNT (76% 2,4-DNT, 19% 2,6-DNT) in the diet after 1 year of treatment, while females had a 50% incidence (CIIT, 1982) (see Table 5-6). Rats treated with 0, 3.5 and 14 mg/kg/day technical grade DNT in the diet and sacrificed after 78 and 104 weeks showed dose-dependent increases in these and other lesions including hepatic neoplastic nodules, testicular mesotheliomas, mammary gland fibroadenomas and adenomas, and subcutaneous fibromas.

Recent investigations utilizing initiation/promotion protocols in rat liver and mouse skin have strongly indicated that 2,6-DNT is the isomer most responsible for the carcinogenic response observed in the above studies (Popp and Leonard, 1982; Leonard et al., 1983; Slaga et al., 1985). These studies have further suggested that both 2,4- and 2,6-DNT are able to enhance the formation of neoplasms. Popp and Leonard (1982) briefly reported that dietary intake of 7 and 14 2,6-DNT/kg/day resulted in a 100 and 95%, respectively, incidence of hepatocellular carcinomas, while a dose of 27 mg 2,4-DNT/kg/day was ineffective at producing tumors. There is therefore a strong possibility that 2,6-DNT is the primary carcinogen in technical grade DNT and it may also contribute to the effects observed in the studies using 2,4-DNT as a contaminant representing 2-5% of the administered dose.

There was no indication of any teratogenic potential for technical grade DNT in rats (Price et al., 1985) or 2,4-DNT in rats (Ellis et al., 1979) or mice (Smith, 1983) at doses that did not produce significant maternal toxicity. In the most comprehensive teratologic study (Price et al., 1985), evidence of embryo/fetal toxicity was cbserved only at a dose (150 mg/kg/day technical grade DNT) that also produced 46.2% maternal mortality.

A significant reduction was reported in the sperm counts of workers exposed to DNT compared with nonexposed workers, although the control values were unusually high (Ahrenholz, 1980). A similar, but more vigorous study was unable to find differences between exposed and control groups in fertility, testicular volume, sperm count and sperm morphology (Hamill et al., 1982). Testicular degeneration and decreased spermatogenesis have been reported to occur following subchronic and chronic exposure to 2,4-DNT in rats (Lee et al., 1985) mice (Hong et al., 1985) and dogs (Ellis et al., 1985). Similar effects were observed in 13-week studies using 2,6-DNT in mice, rats and dogs (Lee et al., 1976).

Treatment of male and female mice with up to 34 and 45 mg/kg/day of 2,4-DNT, respectively, for 6 months did not result in any significant changes in fertility or reproductive performance in rats (Ellis et al., 1979). An assessment of the data generated by genetic and reproductive toxicity studies suggests that while 2,4- and 2,6-DNT can produce testicular damage, they do not appear to be genetically active in male germ cells. The data are currently insufficient to assess the reproductive hazard of the DNTs in females.

Chronic and subchronic toxicity studies have revealed that the blood, liver, testis and neuromuscular system are the primary organs affected by the DNTs. Decreases in average body weight gain were the only significant effects observed in mice and rats exposed to relatively low levels of 2,4-DNT (0.04% and 0.02%, respectively) in the diet in a 2-year NCI (1978) bioassay. The major adverse effect in dogs treated with 2,4-DNT for 13 weeks or 2 years was a neuropathy characterized by incoordination and paralysis. Central nervous system lesions were also seen in some affected dogs given 1.5 mg/kg/day for 2 years, 10 mg/kg/day for 6 months and 25 mg/kg/day for 2 months. Less important adverse effects included methemoglobinemia and its sequelae, testicular atrophy and biliary tract hyperplasia (Ellis et al., 1985).

In mice, 2,4-DNT is apparently more toxic to males than females. Feeding 2,4-DNT in the diet for 13 weeks at concentrations of 0.07 and 0.2% resulted in decreased weight gain in males but not females (Hong et al., 1985). A high dose of 0.7% resulted in depressed body weight, mild anemia and mild hepatocellular dysplasia in both sexes as well as moderate testicular atrophy in males. Chronic exposure of mice to dietary concentrations of 0.01, 0.07 or 0.7% 2,4-DNT resulted in a high incidence of epithelial renal tumors and hepatocellular dysplasia in all treated males, testicular atrophy in middle- and high-dose males and toxic anemia and increased mortality in the high-dose male group. Serious adverse effects in females occurred primarily in the high-dose group and consisted of toxic anemia, hepatocellular dysplasia, nonfunctioning follicles with a lack of corpora lutea and death. In addition, a generalized pigmentation, most likely related to 2,4-DNT, was observed in several tissues in all treated mice, especially in the high-dose groups.

Treatment of rats with 2,4-DNT in the diet at concentrations of 0.07, 0.2 or 0.7% for 13 weeks caused a depression in weight gain in all low-dose animals and weight loss in middle and high-dose rats (Lee et al., 1985).

Relative changes in organ weights, decreased spermatogenesis, reticulocytosis and splenic hemosiderosis, and demyelination in the cerebellum and brainstem were observed in middle- and high-dose rats. In addition, high-dose males and females were anemic and had increased mortality. Chronic treatment of rats for 2 years suggested that a dietary concentration of 0.0015% produced no apparent toxicity. A dose of 0.01% in the diet, however, was moderately toxic while a concentration of 0.07% was quite toxic and shortened life span. These dose levels were associated with a progressive development of hepatic hyperplastic foci to hepatocellular carcinomas, atrophy of seminiferous tubules and decreased spermatogenesis, an increased incidence of subcutaneous tumors, toxic anemia with reticulocytosis and indications of mild neuromuscular effects (Lee et al., 1985). Feeding rats a diet containing a relatively high concentration of 2,4-DNT (0.5%) for 6 months resulted in a significant weight loss, a high rate of mortality, hepatic hypertrophy, testicular atrophy and pronounced methemoglobinemia (Kozuka et al., 1979).

A study by CIIT (1982) reported the effects of feeding technical grade DNT to rats for 104 weeks at dose levels of 3.5, 14 or 35 mg/kg/day. General signs of toxicity including decreased weight gain, alterations in the relative weights of several organs and various histologic tissue alterations occurred in all dose groups of both sexes. In the middle- and high-dose groups, degenerative and proliferative alterations of the hepatocytes and bile duct epithelium were observed within the first 26 weeks of treatment. These alterations generally involved necrotic or vacuolated hepatocytes and necrosis and hyperplasia of the biliary epithelium. These lesions were generally more prevalent in males, were dose-related, and progressed throughout the life of the study. A high incidence of hepatotoxicity, and hepatic neoplasms, mammary fibroadenomas and subcutaneous fibromas, neurofibromas and sarcomas were also observed. In addition, a low grade regenerative anemia occurred in the high-dose groups.

The subchronic toxicity of 2,6-DNT has been discussed only in an abstract with very few details provided (Ellis et al., 1976). The effects in dogs, mice and rats were reportedly similar to those seen in short-term studies using 2,4-DNT (Hong et al., 1985; Lee et al., 1985; Ellis et al., 1985). Doses of 4, 20 or 100 mg/kg/day for dogs and 0.01, 0.05 or 0.25% in the diet for rats and mice exerted effects primarily on the red cells (toxic anemia), nervous system (incoordination, demyelination) and testes (atrophy and decreased spermatogenesis). Other affects included weight loss or decreased weight gain and increased mortality.

The acute toxic effects of DNT most commonly include central nervous system depression, respiratory depression and ataxia. Oral LD_{50} values range from 177 mg/kg for 2,6-DNT in rats (Vernot et al., 1977) to 1954 mg/kg for 2,4-DNT in male mice (Ellis et al., 1980). Generally, the data suggest that mice and rats were more susceptible to the acute effects of 3,5-DNT compared with the other isomers. Mice appeared somewhat more resistant to the effects of all DNT isomers compared with rats (Ellis et al., 1980). Cats are especially sensitive, having an oral LD_{Lo} of 27 mg/kg for 2,4-DNT (Spector, 1956). Eye and skin irritation effects for all isomers are, at most, minimal (Ellis et al., 1980).

In humans, a number of signs and symptoms including headache, vertigo, fatigue, dizziness, weakness, nausea, vomiting, dyspnea, drowsiness, arthralgia, insomnia, tremor, paralysis, unconsciousness, chest pain, shortness of breath, palpitation, anorexia, weight loss and an unpleasant metallic taste in the mouth reportedly resulted from 2,4-DNT exposure

(Floret, 1929; Lewin, 1921; McGee et al., 1942; Perkins, 1919; TSCA-ITC, 1981; U.S. EPA, 1980a). Medical examinations of 2,4-DNT-exposed workers indicated impaired reflexes, nystagmus, tremors, pallor, cyanosis, anemia, leukocytosis, hypertension, dermatitis, leukopenia and hepatitis or jaundice (Floret, 1929; McGee et al., 1942). Improved plant hygiene and worker awareness apparently lessened or eliminated many of these effects (McGee et al., 1947).

6. AQUATIC TOXICITY

6.1. ACUTE

Results of studies comparing acute toxicity of DNT isomers with various aquatic organisms are summarized in Table 6-1. In a static 96-hour bioassay with fathead minnows, Pimephales promelas, 2,3-, 2,5- and 3,4-DNT were ~10 times more toxic (96-hour LC_{50} = 1.3-1.8 mg/ℓ) than 2,4-, 2,6- and 3,5-DNT (96-hour) (LC_{50} = 21.8-47.5 mg/ℓ) (Liu et al., 1983b). Acute toxicity levels determined in other studies generally fall in the same range. U.S. EPA (1980a) and Hartley (1981) reported 96-hour LC_{50} values for 2,4-DNT to fathead minnows of 31 mg/ℓ and 34.7 mg/ℓ, respectively.

Buccafusco et al. (1981) reported 24- and 96-hour LC_{50} values of 1.6 and 0.33 mg/ℓ 2,3-DNT, respectively, for bluegills, Lepomis macrochirus, in a static bioassay. Hartley (1981) reported LC_{50} values for 2,4-DNT to various aquatic species (Table 6-2). He found that the toxicity of 2,4-DNT to bluegills was strongly temperature-dependent, with 96-hour LC_{50} values of 23.2 mg/ℓ at 17°C and 8.6 mg/ℓ at 24°C. Toxicity was not affected by water hardness, however. In tests with channel catfish, Ictalurus punctatus, and fathead minnows, Hartley (1981) found little difference in LC_{50} values determined in either static or flow through bioassays (see Table 6-2). Of the fish species tested by Hartley (1981), bluegills and rainbow trout, Salmo gairdneri, appeared to be the most sensitive to 2,4-DNT. Adema et al. (1983) determined a 96-hour LC_{50} of 25 mg/ℓ 2,4-DNT for guppies, Poecilia reticulata, and a NOEC of 3.2 mg/ℓ. Jaffe et al. (1973) reported the results of a study in which bluegills were exposed to an unspecified mixture of DNT isomers. The 24-, 48- and 96-hour LC_{50} values were 50, 27 and 16 mg/ℓ, respectively.

TABLE 6-1

Comparison of Acute Toxicity Parameters for DNT Isomers in Aquatic Organisms

Species	Parameter	DNT Isomer						Reference
		2,3	2,4	2,5	2,6	3,4	3,5	
Fathead minnow *Pimephales promelas*)	96-hour LC_{50}	1.8	32.8	1.3	18.5	1.5	22.6	Liu et al., 1983b
Water flea *Daphnia magna*)	48-hour EC_{50}	4.7	47.5	3.1	21.8	3.7	45.2	Liu et al., 1983b
Water flea *Daphnia magna*)	24-hour LC_{50}	3.2	22	14	NT	NT	NT	Bringmann and Kuehn, 1977
Water flea *Daphnia magna*)	EC_{50}	3.9	22	21	NT	NT	NT	Bringmann and Kuehn, 1982
Flagellate *Entosiphon sulcatum*)	toxicity threshold	5.9	0.98	11	NT	NT	NT	Bringmann and Kuehn, 1981
Flagellate *Chilomonas paramaecium*)	toxicity threshold	1.8	0.13	>20	NT	NT	NT	Bringmann and Kuehn, 1981
Ciliate *Uronema parduczi*)	toxicity threshold	1.6	0.55	23	NT	NT	NT	Bringmann and Kuehn, 1981
BG algae *Microcystis aeruginosa*)	toxicity threshold	0.22	0.13	0.5	NT	NT	NT	Bringmann and Kuehn, 1978
Algae *Scenedesmus quadricauda*)	toxicity threshold	0.83	2.7	12	NT	NT	NT	Bringmann and Kuehn, 1978
Bacteria *Pseudomonas putida*)	toxicity threshold	9	57	26	NT	NT	NT	Bringmann and Kuehn, 1980

NT = Not tested

TABLE 6-2

Acute Toxicity of 2,4-DNT to Aquatic Organisms*

Species	Exposure	Duration	LC_{50} (mg/ℓ)
Bluegill	static	96 hours	15.7
Bluegill	static, 17°C	96 hours	23.2
Bluegill	static, 21°C	96 hours	12.8
Bluegill	static, 24°C	96 hours	8.6
Fathead minnow	static	96 hours	34.6
Fathead minnow	flowthrough	96 hours	36.5
Fathead minnow	flowthrough	14 days	26.1
Channel catfish	static	96 hours	28.8
Channel catfish	flowthrough	96 hours	29.9
Channel catfish	flowthrough	14 days	15.6
Rainbow trout	flowthrough	96 hours	14.0
Rainbow trout	flowthrough	14 days	6.8
Oligochaete _Lumbriculus_ _variegatus_	flowthrough	24 hours 96 hours 14 days	99.5 50.7 31.1
Midge _Tanytarsus_ sp.	static	48 hours	22.5
Water flea _Daphnia_ sp.	static	48 hours	38.3

*Source: Hartley, 1981

The only data for marine fish species were provided by Heitmuller et al. (1981) who determined 24-, 48-, 72- and 96-hour LC_{50} values of >7.5, 5.0, 2.9 and 2.3 mg/ℓ 2,3-DNT for sheepshead minnows, Cyprinodon variegatus.

Various authors have investigated differences in toxicity of various DNT isomers to invertebrate species (see Table 6-1). Liu et al. (1983b) found the same pattern in 48-hour EC_{50} values for Daphnia magna as they did in fathead minnows; i.e., the 2,3-, 2,5- and 3,4-isomers were ~10 times more toxic (EC_{50} = 3.1-4.7 mg/ℓ) than the 2,4-, 2,6- and 3,5-isomers (EC_{50} = 21.8-47.5 mg/ℓ). In a study using fewer DNT isomers, Bringmann and Kuehn (1977) also found that 2,3-DNT was more toxic (24-hour LC_{50} = 3.2 mg/ℓ) than 2,4-DNT (22 mg/ℓ) or 2,6-DNT (14 mg/ℓ) to Daphnia magna. Similar results were obtained in a study where the EC_{50} (for immobilization) was used as the response variable rather than the LC_{50} (see Table 6-1) (Bringmann and Kuehn, 1982). U.S. EPA (1980a) reported Daphnia magna 48-hour EC_{50} values of 0.66 and 35.0 mg/ℓ for 2,3- and 2,4-DNT, respectively.

Other values for acute toxicity of individual DNT isomers to aquatic invertebrates have been reported. Hartley (1981) reported 48-hour LC_{50} values of 38.3 and 22.5 mg/ℓ 2,4-DNT to water flea, Daphnia, and midges, Tanytarsus, respectively, under static conditions. In a similar bioassay, the oligochaete worm, Lumbriculus variegatus, and the amphipod, Hyallella azteca, were apparently unaffected by 48-hour exposures to 83.2 mg/ℓ. Hartley (1981) also conducted a flowthrough bioassay using Lumbriculus variegatus and found that it was less sensitive to 2,4-DNT than to fishes under similar conditions, with 24-hour, 96-hour and 14-day LC_{50} values of 99.5, 50.7 and 31.1 mg/ℓ, respectively (see Table 6-2). Adema et al. (1983) determined a 2-day EC_{50} for immobilization of Daphnia magna to be

>10 but <16 mg/ℓ 2,4-DNT. The NOEC was 10 mg/ℓ. LeBlanc (1980) determined a 48-hour LC_{50} of 0.66 mg/ℓ 2,3-DNT for *Daphnia magna*, and a NOEC of <0.046 mg/ℓ. The only information for marine invertebrates was provided by LeBlanc (1984) who reported a 48-hour LC_{50} of 0.59 mg/ℓ 2,3-DNT to mysid shrimp, *Mysidopsis gahia*.

Bringmann and Kuehn (1981) determined "toxicity thresholds" for inhibition of cell multiplication of various protozoans by DNT isomers (see Table 6-1). In contrast to other organisms, the three protozoans, *Entosiphon subcatum*, *Chilomonas paramaecium* and *Uronema parduczi*, were most sensitive to 2,4-DNT (toxicity threshold = 0.13-0.98 mg/ℓ) rather than 2,3-DNT (1.5-5.9 mg/ℓ) or 2,6-DNT (11-23 mg/ℓ).

6.2. CHRONIC

Hartley (1981) reported results of a chronic bioassay with juvenile fathead minnows. A concentration of 7 mg/ℓ 2,4-DNT significantly reduced the number of viable fry emerging from eggs. Concentrations of 1.75-7.0 mg/ℓ 2,4-DNT had a significant effect on 30-day fry length. Exposure to 7 mg/ℓ 2,4-DNT for 90-150 days adversely affected survival.

Hartley (1981) conducted 8-week studies with bluegills exposed to 2,4-DNT at concentrations that were 0-60% of the 96-hour LC_{50}, 12.8 mg/ℓ. Growth was completely inhibited at 5 and 8 mg/ℓ, and was depressed at 2 mg/ℓ. Growth was unaffected in fish exposed to 0.5 mg/ℓ. In a subsequent study using lower concentrations, Hartley (1981) concluded that the threshold for growth depression was 0.05 mg/ℓ 2,4-DNT, with a dose-related increase in growth depression up to 5 mg/ℓ. Histopathological analyses in these studies revealed no abnormalities in tissues of fish exposed to 0.05, 0.5, 1 or 2 mg/ℓ. Fish exposed to 4, 5 and 8 mg/ℓ experienced fatty changes in liver, with some necrotic foci occurring in fish at the high concentration. Severe gill epithelial hyperplasia and some kidney necrosis also occurred in fish exposed to 5 or 8 mg/ℓ.

U.S. EPA (1980a) reported the results of a fathead minnow embryo-larval bioassay. The chronic value derived from this study, which was based on survival, was 0.230 mg/ℓ.

Adema et al. (1983) determined that 1.0 mg/ℓ 2.4-DNT was a NOEC for effects on egg-larval development, mortality, growth, swimming behavior and color of flagfish, *Jordanella florialae*, in a 6-week study. In separate experiments, they determined NOECs of 1.8 and 3.2 mg/ℓ 2,4-DNT for effects on growth, mortality, swimming behavior and color of guppies, *Poecilia reticulata*, in 4-week studies. In separate 3-week studies, 0.32 and 1.0 mg/ℓ 2,4-DNT were found to be NOECs for *Daphnia magna*. Effects of concern were mortality, reproduction and growth (Adema et al., 1983).

6.3. PLANTS

Various authors have tested DNT isomers for toxicity to algae. Bringmann and Kuehn (1978) determined toxicity thresholds of DNT isomers for inhibition of cell multiplication in algal cultures. The green alga, *Scenedesmus quadricauda*, was affected by 2,3-DNT (toxicity threshold = 0.83 mg/ℓ) more than by 2,4-DNT (toxicity threshold = 2.7 mg/ℓ), while 2,6-DNT was least toxic (toxicity threshold = 12 mg/ℓ). In control, the three isomers were about equally toxic to the blue-green alga *Microcystis aeruginosa* (toxicity threshold = 0.13 - 0.50 mg/ℓ) (see Table 6-1). Adema et al. (1983) determined an EC_{50} of 2.3 mg/ℓ 2,4-DNT and a NOEC of 0.32 mg/ℓ for inhibition of cell multiplication in *Scenedesmus pannonicus*. Hartley (1981) reported 14-day EC_{50} values (for an unspecified effect) of 3.3, 8 and 10.2 mg/ℓ 2,4-DNT for *Selenastrum capricornutum*, *Navicula pelliculosa* and *Anabena* sp., respectively.

Bringmann and Kuehn (1980) reported that the bacterium *Pseudomonas putidum* was less sensitive than algae species to cell multiplication inhibition by DNT isomers. The most toxic isomer was 2,3-DNT (toxicity threshold = 9 mg/ℓ); 2,6-DNT was intermediate (toxicity threshold = 26 mg/ℓ) and 2,4-DNT was least toxic (57 mg/ℓ) (see Table 6-1).

There is little information available concerning toxicity of DNT to aquatic macrophytes. U.S. EPA (1978) reported that concentrations of ≥1 mg/ℓ 2,4-DNT depressed or killed colonies of duckweed, *Lemna perpusilla*. Another report cited a NOEL of 0.1-0.5 mg/ℓ 2,4-DNT for growth inhibition of the same species (TSCA-ITC, 1981).

6.4. RESIDUES

Hartley (1981) reported that the 4-day BCFs for bluegills exposed to 1 mg/ℓ 2,4-DNT were 78 in viscera and 4 in muscle. After 3 days of depuration, virtually all of the 2,4-DNT was eliminated. Hartley (1981) conducted an experiment in which bluegills were exposed to 3 mg/ℓ 2,4-DNT for 14 days. BCFs for various tissues are reported in Table 6-3. The highest uptake and elimination rate occurred in the brain. The concentration of 2,4-DNT in fish striated muscle is important because of possible human exposure through eating contaminated fish. Muscle showed the lowest 2,4-DNT accumulation of any tissue examined. Concentrations of 2,4-DNT in muscle and other tissues were again reduced to zero after 3-4 days in 2,4-DNT-free water.

Liu et al. (1983b) reported that the 4-day BCFs for 2,4-DNT were 78 in bluegill viscera and for the oligochaete, *Lumbriculus variegatus*, and 13 for *Daphnia magna*. The authors determined a 4-day BCF of 2507 for 2,4-DNT in the alga, *Selenastrum capricornutum*, but noted that this may have been due to sorption of 2,4-DNT to the outer walls of the cells rather than actual

TABLE 6-3

14-Day BCFs for Tissues of Bluegills Exposed to 3 mg/ℓ 2,4-DNT*

Tissue	Maximum 2,4-DNT Concentration in Tissue (μg/g)	BCF
Whole body	72	25
Brain	199	103
Kidney	140	48
Stomach and intestine	85	29
Liver	85	29
Gill	69	24
Striated muscle	31	11

*Source: Hartley, 1981

uptake. BCFs for 2,4-DNT were slightly higher for all organisms except the alga _S_. _capricornutum_ when 2,4-DNT was administered as part of mixture of other chemicals. Liu et al. (1983b) calculated steady-state BCF values based on estimated K_{ow}s. These calculated BCFs were 31.83 for all DNT isomers. The available information indicates that 2,4-DNT does not accumulate appreciably in fish, and this is probably true of other DNT isomers as well (Bailey and Spanggord, 1983; Liu et al., 1983b).

6.5. SUMMARY

The 2,3-, 2,5- and 3,4-isomers of DNT are apparently more toxic to fish (fathead minnows) and cladocerans (_Daphnia magna_) than the 2,4-, 2,5- and 3,5-isomers (Liu et al., 1983b). The 2,4-isomer is more toxic to protozoans than the 2,3- or 2,6-isomers (Bringmann and Kuehn, 1981). Algae and protozoa appear to be more sensitive to 2,4-DNT toxicity than fish and invertebrates, with acute toxicity occurring at levels of 0.13-2.7 mg/ℓ, compared with 22-47.5 mg/ℓ for fathead minnows and _Daphnia magna_ (see Table 6-1).

Among fish species, bluegills and rainbow trout are more sensitive to 2,4-DNT than channel catfish and fathead minnows (Hartley, 1981). Based on the available data, U.S. EPA (1980a) concluded that acute and chronic toxicity to freshwater biota occurred at DNT concentrations as low as 0.330 and 0.230 mg/ℓ, respectively, and that acute toxicity to saltwater organisms occurred at concentrations as low as 0.590 mg/ℓ. More recent data indicating lower adverse effect concentrations than these could not be located in the available literature. The limited information on DNT residues indicates that DNT isomers are not strongly bioaccumulated by aquatic organisms (Bailey and Spanggord, 1983; Hartley, 1981; Liu et al., 1983b), and therefore biomagnification and human exposure from contaminated fish should not be a major concern.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

ACGIH (1985) adopted a TWA-TLV for DNT of 1.5 mg/m^3. This value was adopted by analogy to the limits established for nitro- and dinitrobenzenes (ACGIH, 1980). This recommendation is accompanied by the "skin" notation indicating that dermal absorption is a potentially hazardous route of exposure.

Based on the induction of mammary and liver tumors in female CD rats fed various doses of 2,4-DNT for 24 months (Lee et al., 1978; Ellis et al., 1979), the U.S. EPA (1980a) has calculated that an ambient water concentration of 1.1 µg/ℓ of 2,4-DNT will result in an individual lifetime cancer risk below 10^{-5}.

7.2. AQUATIC

U.S. EPA (1980a) reportedd that acute and chronic toxicity to freshwater aquatic life occurred at DNT concentrations as low as 330 and 230 µg/ℓ, respectively, and would occur at lower concentrations in species more sensitive than those tested. U.S. EPA (1980a) also reported that acute toxicity to saltwater organisms occurred at concentrations as low as 590 µg/ℓ. No information regarding chronic toxicity to marine organisms was presented, although it was noted that algal cell numbers were decreased by concentrations as low as 370 µg/ℓ.

8. RISK ASSESSMENT

Several bioassays have been performed regarding the carcinogenicity of DNT and individual DNT isomers. Rats exposed to 80 and 200 ppm 2,4-DNT in the diet for 78 weeks were reported to have a significant dose-related increased incidence of subcutaneous fibromas in males and statistically significant increased frequency of mammary gland fibroadenomas in high-dose females compared with controls (see Table 5-1). Male and female mice exposed to 80 and 400 ppm in the diet for 78 weeks displayed no evidence of a significant tumor incidence in any tissue relative to control groups (NCI, 1978).

Administration of 2,4-DNT to rats at dietary concentrations of 0, 15, 100 and 700 ppm for 2 years resulted in an increase in the number of high-dose rats with hepatocellular carcinomas that was statistically significant only in females (Ellis et al., 1979). High-dose females also had a 97% incidence of benign mammary gland neoplasms (see Table 5-2). Ellis et al. (1979) also reported a statistically significant increase in the number of high-dose male mice with benign (cystic papillary adenomas) and malignant (solid renal cell carcinomas, cystic papillary carcinomas) renal tumors following dietary administration of 0, 100 or 700 ppm 2,4-DNT. Hepatomas were also observed in these mice but the incidence was not dose-related (see Table 5-3). Female mice maintained on the same diet had no treatment-related increase in tumor frequency in any tissue.

A 100% incidence of hepatocellular carcinomas was reported in male rats exposed to 35 mg/kg/day technical grade DNT (76% 2,4-DNT, 19% 2,6-DNT) in the diet after 1 year of treatment, while females had a 50% incidence (CIIT, 1982). Rats treated with 0, 3.5 and 14 mg/kg/day technical grade DNT in the diet and sacrificed after 78 and 104 weeks showed dose-related increases in these and other lesions including hepatic neoplastic nodules (see Table 5-4), testicular mesotheliomas, mammary gland fibroadenomas and adenomas and subcutaneous fibromas.

Recent investigations utilizing initiation/promotion protocols in rat liver and mouse skin have strongly indicated that 2,6-DNT is the isomer most responsible for the carcinogenic response observed in the above studies (Popp and Leonard, 1982; Leonard et al., 1983; Slaga et al., 1985). These studies have further suggested that both 2,4- and 2,6-DNT are able to enhance the formation of neoplasms. Popp and Leonard (1982) briefly reported that dietary intake of 7 and 14 mg 2,6-DNT/kg/day resulted in a 95 and 100%, respectively, incidence of hepatocellular carcinomas, while a dose of 27 mg 2,4-DNT/kg/day was ineffective at producing tumors. There is therefore a strong possibility that 2,6-DNT is the primary carcinogen in technical grade DNT, and it may also contribute to the effects observed in the studies using 2,4-DNT as a contaminant representing 2-5% of the administered dose.

Although many assays regarding the mutagenicity of the DNTs have been performed, the results are not consistent (see Table 5-5).

There was no indication of any teratogenic potential for technical grade DNT in rats (Price et al., 1985) or 2,4-DNT in rats (Ellis et al., 1979) or mice (Smith, 1983) at doses that did not produce significant maternal toxicity. In the most comprehensive teratologic study (Price et al., 1985), evidence of embryo/fetal toxicity was observed only at a dose (150 mg/kg/day technical grade DNT) that also produced 46.2% maternal mortality.

A significant reduction in the sperm counts of workers exposed to DNT compared with nonexposed workers was reported, although the control values were unusually high (Ahrenholz, 1980). A similar, but more rigorous study found no differences between exposed and control groups in regards to fertility, testicular volume, sperm count and sperm morphology (Hamill et al., 1982). Testicular degeneration and decreased spermatogenesis have been reported to occur following subchronic and chronic exposure to 2,4-DNT in rats (Lee et al., 1985) mice (Hong et al., 1985) and dogs (Ellis et al., 1985). Similar effects were observed in 13-week studies using 2,6-DNT in mice, rats and dogs (Lee et al., 1976).

Treatment of male and female mice with up to 34 and 45 mg/kg/day of 2,4-DNT, respectively, for 6 months did not result in any significant changes in fertility or reproductive performance in rats (Ellis et al., 1979). An assessment of the data generated by genetic and reproductive toxicity studies suggests that while 2,4- and 2,6-DNT can produce testicular damage, they do not appear to be genetically active in male germ cells. The data are currently insufficient to assess the reproductive hazard of the DNTs in females.

Chronic and subchronic toxicity studies have revealed that the blood, liver, testis and neuromuscular system are the primary organs affected by the DNTs. Decreases in average body weight gain were the only significant effects observed in mice and rats, exposed to relatively low levels of 2,4-DNT (0.04% and 0.02%, respectively) in the diet in a 2-year NCI (1978) bioassay. The major adverse effect in dogs treated with 2,4-DNT for 13 weeks or 2 years was a neuropathy characterized by incoordination and paralysis (Ellis et al., 1985). Central nervous system lesions were also seen in some affected dogs given 1.5 mg/kg/day for 2 years, 10 mg/kg/day for 6 months and 25 mg/kg/day for 2 months. Less important adverse effects included methemoglobinemia and its sequelae, testicular atrophy and biliary tract hyperplasia.

In mice, 2,4-DNT is apparently more toxic to males than females. Feeding 2,4-DNT in the diet for 13 weeks at concentrations of 0.07 and 0.2% resulted in decreased weight gain in males but not females (Hong et al., 1985). The high level of 0.7% resulted in depressed body weight, mild anemia and mild hepatocellular dysplasia in both sexes as well as moderate testicular atrophy in males. Chronic exposure of mice to dietary concentrations of 0.01, 0.07 or 0.7% 2,4-DNT resulted in a high incidence of epithelial renal tumors and hepatocellular dysplasia in all treated males, testicular atrophy in middle- and high-dose males and toxic anemia and increased mortality in the high-dose male group. Serious adverse effects in females occurred primarily in the high-dose group and consisted of toxic anemia, hepatocellular dysplasia, nonfunctioning follicles with a lack of corpora lutea and death. In addition, a generalized pigmentation, most likely related to 2,4-DNT, was observed in several tissues in all treated mice, especially in the high-dose groups.

Treatment of rats with 2,4-DNT in the diet at concentrations of 0.07, 0.2 or 0.7% for 13 weeks caused a depression in weight gain in all low-dose animals and weight loss in middle and high-dose rats (Lee et al., 1985). Relative changes in organ weights, decreased spermatogenesis, reticulocytosis and splenic hemosiderosis, and demyelination in the cerebellum and brainstem were observed in middle- and high-dose rats. In addition, high-dose males and females were anemic and had increased mortality. Chronic treatment of rats for 2 years suggested that a dietary concentration of 0.0015% produced no apparent toxicity. A middle dose of 0.01% in the diet, however, was moderately toxic, while a concentration of 0.07% was quite toxic and shortened the life span. These dose levels were associated with a progressive development of hepatic hyperplastic foci to hepatocellular carcinomas, atrophy of seminiferous tubules and decreased spermatogenesis, an increased incidence of subcutaneous tumors, toxic anemia with reticulocytosis and indications of mild neuromuscular effects. Feeding rats a diet containing a relatively high concentration of 2,4-DNT (0.5%) for 6 months resulted in a significant weight loss, a high rate of mortality, hepatic hypertrophy, testicular atrophy and pronounced methemoglobinemia (Kozuka et al., 1979).

A study by CIIT (1982) reported the effects of feeding technical grade DNT to rats for 104 weeks at dose levels of 3.5, 14 or 35 mg/kg/day. General signs of toxicity including decreased weight gain, alterations in the relative weights of several organs and various histologic tissue alterations occurred in all dose groups of both sexes. In the middle- and high-dose groups, degenerative and proliferative alterations of the hepatocytes and bile duct epithelium were observed within the first 26 weeks of treatment. These alterations generally involved necrotic or vacuolated hepatocytes and necrosis and hyperplasia of the biliary epithelium. These lesions were generally more prevalent in males, were dose-related and progressed throughout the life of the study. A high incidence of hepatotoxicity, and hepatic neoplasms, mammary fibroadenomas and subcutaneous fibromas, neurofibromas and sarcomas were also observed. In addition, a low grade regenerative anemia occurred in the high-dose groups.

The subchronic toxicity of 2,6-DNT has been discussed only in an abstract with very few details provided (Ellis et al., 1976). The effects in dogs, mice and rats were reportedly similar to those seen in short-term studies using 2,4-DNT (Hong et al., 1985; Lee et al., 1985; Ellis et al., 1985). Oral doses of 4, 20 or 100 mg/kg/day for dogs and 0.01, 0.05 or 0.25% in the diet for rats and mice exerted effects primarily on the red cells (toxic anemia), nervous system (incoordination, demyelination) and testes (atrophy and decreased spermatogenesis). Other affects included weight loss or decreased weight gain and increased mortality.

It is apparent from the data presented that technical grade DNTs and the 2,4-isomer are capable of inducing a carcinogenic response in laboratory animals. The data of Ellis et al. (1979) for 2,4-DNT and CIIT (1982) for technical grade DNT have demonstrated dose-related increases in tumor incidences, thus permitting the calculation of q_1* values for humans (Tables 8-1 to 8-5). No q_1* values were calculated for the NCI (1978) study because only the incidence of benign tumors was increased. Although there is evidence that 2,6-DNT is carcinogenic (Popp and Leonard, 1982; Leonard et al., 1983), the published data are insufficient to derive a q_1* because the number of experimental rats was not specified.

Because the report by Ellis et al. (1979) provided pathology data for individual animals, it was possible to combine tumor incidences. Female rats exposed to 0, 15, 100 or 700 ppm 2,4-DNT in the diet for 2 years had a combined mammary and liver tumor incidence of 11/23, 12/35, 17/27 and 34/35, respectively (Ellis et al., 1979). These dose levels corresponded to average 2,4-DNT intake values of 0, 0.706, 5.14 and 45.3 mg/kg/day, respectively, based on average monthly measurements of feed consumption. Because the weights of the rats in different treatment groups varied, each dose was transformed to the corresponding human dose before calculating the q_1* by multiplying the rat dose by the cube root of the ratio of the rat body weight to the reference human (70 kg) body weight (U.S. EPA, 1980b) (see Table 8-1). From these doses, a human q_1* of 6.83×10^{-1} $(mg/kg/day)^{-1}$ was calculated using the computerized multistage model developed by Howe and

TABLE 8-1

Cancer Data Sheet for Derivation of q_1*

Compound: 2,4-DNT

Reference: Ellis et al., 1979

Species, Strain, Sex: rat, Sprague-Dawley, female

Body weight: 0.425 kg control; 0.425 kg low dose; 0.410 kg middle dose; 0.325 kg high dose (measured)

Length of exposure (le) = 24 months

Length of experiment (Le) = 24 months

Lifespan of animal (L) = 24 months

Tumor site and type: liver - hepatocellular carcinoma, neoplastic nodule
mammary gland - adenoma, fibroadenoma, fibroma, adenocarcinoma-carcinoma

Route, vehicle: oral, diet

Experimental Doses or Exposures		Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Examined
(ppm)	(mg/kg/day)		
0	0	0	11/23
15	0.706	0.129	12/35
100	0.514	0.927	17/27
700	45.3	7.557	34/35

Unadjusted q_1* from study = not calculated (see text)

Human q_1* = 6.8301346×10^{-1} $(mg/kg/day)^{-1}$

TABLE 8-2

Cancer Data Sheet for Derivation of q_1^*

Compound: 2,4-DNT

Reference: Ellis et al., 1979

Species, Strain, Sex: mouse, Swiss, male

Body weight: 0.04 kg (measured)

Length of exposure (le) = 24 months

Length of experiment (Le) = 24 months

Lifespan of animal (L) = 24 months

Tumor site and type: kidney; cystic papillary adenoma, cystic papillary carcinoma, solid renal cell carcinoma

Route, vehicle: oral, diet

Experimental Doses or Exposures (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Examined
0	0	0/20
100	13.3	4/21
700	96.9	16/17

Unadjusted q_1^* from study = 3.2460682×10^{-2} $(mg/kg/day)^{-1}$

Human q_1^* = 3.9117431×10^{-1} $(mg/kg/day)^{-1}$

TABLE 8-3

Cancer Data Sheet for Derivation of q_1*

Compound: practical grade 2,4-DNT

Reference: Lee et al., 1978

Species, Strain, Sex: rat, CD, female

Body weight: 0.464 kg

Length of exposure (le) = 720 days

Length of experiment (Le) = 750 days

Lifespan of animal (L) = 750 days

Transformed Dose† (mg/kg/day)	Incidence (No. responding/No. tested)
0.0	11/31
0.75	13/43
5.0	18/35
35.0	35/43

†As reported in U.S. EPA, 1980a, 1981

Human q_1* = 0.31 $(mg/kg/day)^{-1}$

TABLE 8-4

Cancer Data Sheet for Derivation of q_1*

Compound: technical DNT

Reference: CIIT, 1982

Species, Strain, Sex: rat, Fischer 344, male

Body weight: 0.37 kg control; 0.35 kg low dose; 0.29 kg high dose; (measured)

Length of exposure (le) = 104 weeks

Length of experiment (Le) = 104 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: liver; hepatocellular carcinoma, neoplastic nodule

Route, vehicle: oral, diet

Experimental Doses or Exposures (mg/kg/day)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Examined
0	0	10/61
3.388	0.579	19/70
13.451	2.160	23/23

Unadjusted q_1* from study = not calculated (see text)

Human $q_1^* = 2.3089356 \times 10^{-1}$ (mg/kg/day)$^{-1}$

TABLE 8-5

Cancer Data Sheet for Derivation of q_1^*

Compound: technical DNT

Reference: CIIT, 1982

Species, Strain, Sex: rat, Fischer 344, female

Body weight: 0.26 kg control; 0.24 kg low dose; 0.19 kg high dose; (measured)

Length of exposure (le) = 104 weeks

Length of experiment (Le) = 104 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: liver; hepatocellular carcinoma, neoplastic nodule

Route, vehicle: oral, diet

Experimental Doses or Exposures (mg/kg/day)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Examined
0	0	5/57
3.379	0.510	12/61
13.633	1.902	66/68

Unadjusted q_1^* from study = not calculated (see text)

Human $q_1^* = 2.0675596\times10^{-1}$ (mg/kg/day)$^{-1}$

Crump (1982). Since the length of the experiment (Le) and the lifespan of the animal (L) were approximately the same, there was no need to correct by a factor of $(L/Le)^3$.

In the same study (Ellis et al., 1979), male mice exposed to 0, 100 or 700 ppm 2,4-DNT for 24 months had renal tumor incidences of 0/20, 4/21 and 16/17, respectively. Based on feed consumption data, dose levels of 0, 13.3 and 96.9 mg/kg/day were reported by the authors. Using the model outlined above, a q_1^* for humans of 3.91×10^{-1} $(mg/kg/day)^{-1}$ was derived by multiplying the unadjusted q_1^* by the cube root of the ratio of human body weight (70 kg) to mouse body weight (0.04 kg) (see Table 8-2). No other correction factors were necessary. Lee et al. (1978) reported an oral cancer bioassay of 2,4-DNT in male and female Charles River CD rats and CD-1 mice fed 2,4-DNT for 2 years. The major pathologic findings in the rats included a significant increase of hepatocellular carcinomas ($p=7.1 \times 10^{-6}$) and neoplastic nodules ($p=0.01$) in the liver of females, mammary gland tumors of the female ($p=8.3 \times 10^{-5}$) and the suspicious increase of hepatocellular carcinomas of the liver in males. All of these rat tumors were in high-dose animals. The pathologic finding in the mice was the highly significant ($p=1.5 \times 10^{-7}$) increase of kidney tumors in the males of the middle-dose group.

Based on this study, U.S. EPA (1981) estimated a q_1^* for humans of 0.31 $(mg/kg/day)^{-1}$ for 2,4-DNT. This value is extremely close to the value based on Ellis et al. (1979). The slightly higher potency value from Ellis et al. (1979) of 0.68 $(mg/kg/day)^{-1}$ for female Sprague-Dawley rats is suggested to conservatively represent the potency of 2,4-DNT.

Similar procedures were used to calculate human q_1^*s from tumor data in male and female rats fed technical grade DNT for 2 years (CIIT, 1982).

Both sexes developed hepatic neoplastic nodules and hepatocellular carcinomas following exposure to nominal doses of 0, 3.5 or 14 mg/kg/day of technical grade DNT in the diet. Based on food consumption data, the investigators calculated the actual doses to be 0, 3.388 and 13.451 mg/kg/day for males and 0, 3.379 and 13.633 mg/kg/day for females (see Tables 8-4 and 8-5). The combined incidences of hepatic lesions for the control, low- and high-dose groups was 10/61, 19/70 and 23/23, respectively, in males and 5/57, 12/61 and 66/68, respectively, for females (see Tables 8-4 and 8-5). Because body weights varied significantly between treatment groups in both sexes, unadjusted q_1^*s were not calculated since the doses used to calculate q_1^* were already adjusted to extrapolate from rats to humans by multiplying the dose by the cube root of the ratio of animal weight to human body weight. The resulting human q_1^*s derived from male and female rat data were 2.31×10^{-1} and 2.07×10^{-1} $(mg/kg/day)^{-1}$, respectively.

The five calculated human q_1^* values are summarized in Table 8-6. The higher of the q_1^* values for 2,4-DNT is 6.8×10^{-1} $(mg/kg/day)^{-1}$ and is recommended for estimating the increased lifetime risk of cancer. The concentrations of 2,4-DNT in water associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are 5.1×10^{-4}, 5.1×10^{-5} and 5.1×10^{-6} mg/ℓ, respectively.

Although the study by Popp and Leonard (1982) indicated a potent carcinogenic response in rats treated orally with 2,6-DNT for 1 year, the lack of incidence data precludes calculation of a q_1^*. Because these investigators indicated that the 2,6-DNT in technical DNT used in the CIIT (1982) study was the primary carcinogenic agent, the q_1^* of 2.3×10^{-1} $(mg/kg/day)^{-1}$ for technical grade DNT is recommended for estimating the increased lifetime cancer risk of exposure to 2,6-DNT until a definitive

TABLE 8-6

Summary of Calculated Human q_1*s for DNT

Species	Compound	Sex	q_1* (mg/kg/day)$^{-1}$	Reference
Rat	2,4-DNT	female	3.1×10^{-1}	Lee et al., 1978
Rat	tDNT	male	2.3×10^{-1}	CIIT, 1982
Rat	tDNT	female	2.1×10^{-1}	CIIT, 1982
Rat	2,4-DNT	female	6.8×10^{-1}	Ellis et al., 1979
Mouse	2,4-DNT	male	3.9×10^{-1}	Ellis et al., 1979

bioassay for 2,6-DNT becomes available. The water concentrations associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are 1.5×10^{-3}, 1.5×10^{-4} and 1.5×10^{-5} mg/ℓ, respectively. Data are insufficient for deriving ADIs or q_1*s for the other DNT isomers.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Studies regarding the fetotoxicity, reproductive effects and subchronic and chronic toxicities of DNT were discussed in Sections 5.3., 5.4. and 5.5. and are summarized in Table 9-1. Average body weights, for the most part, were estimated from growth curves or were calculated from compound intake and food consumption data [if the rats consumed 21.6 g/day of a diet containing 0.07% (700 ppm or 700 mg/kg food) 2,4-DNT and the average intake of the compound was 34 mg/kg/day, then the average weight of the rats in that dose group is 0.0216 kg diet/day x 700 mg/kg diet ÷ 34 mg/kg/day = 0.445 kg]. When data were not supplied, as in the studies by Ellis et al. (1976, 1985), reference body weights of 0.35 kg for rats and 0.03 kg for mice, as recommended by U.S. EPA (1980b) and 12.7 kg for dogs, as recommended by Durkin (1985), were used. Transformed animal doses in mg/kg/day (see Table 9-1) were as reported in the studies except for the NCI (1978) and the Ellis et al. (1976) studies in which doses were calculated assuming rats and mice consume food equivalent to 5 and 13%, respectively, of their body weights/day. Equivalent human doses were calculated by multiplying the transformed animal dose by the cube root of the ratio of the animal body weight to the reference human body weight of 70 kg.

It is apparent from Table 9-1 that DNT affects a number of organ systems, primarily the blood (anemia, methemoglobinemia, reticulocytosis), central nervous system (demyelination, paralysis and muscular incoordination), liver (necrosis, degeneration, dysplasia, carcinoma) and gonads (testicular and ovarian atrophy, aspermatogenesis). These effects were generally dose-related and have been observed in dogs, mice and rats given 2,4-, 2,6- and technical grade DNT. Increased mortality, not necessarily

TABLE 9-1

Oral Toxicity Summary of the DNTs

Compound	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Dosage/Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Response	Reference
2,4-DNT	rat/ Fischer 344	M/F	50/50	0.3 (M) 0.225 (F)	diet	0.02% in feed for 78 weeks followed by 26-week observation period	10[a]	1.6 (M) 1.5 (F)	Decreased body weight gain	NCI, 1978
2,4-DNT	mouse/ B6C3F1 344	M/F	50/50	0.035 (M/F)	diet	≥0.008% in feed for 78 weeks followed by 13-week observation period	10.4[a]	0.83	Decreased body weight gain	NCI, 1978
2,4-DNT	dog/ beagle	M/F	4/4	12.7	lactose/ gelatin capsule	25 mg/kg/day for 13 weeks	25	14.2	Neurotoxicity (incoordination, paralysis, histopathologic lesions); testicular degeneration with reduced spermatogenesis	Ellis et al., 1985
2,4-DNT	dog/ beagle	M/F	6/6	12.7	lactose/ gelatin capsule	≥1.5 mg/kg/day for 24 months	1.5	0.85	Neuromuscular dysfunction paralysis, histopathologic lesions in brain; anemia, biliary tract hyperplasia	Ellis et al., 1985
2,4-DNT	rat/CD	M/F	16/16	0.445 (M) 0.273 (F)	diet	0.07% in feed for 13 weeks	34 (M) 38 (F)	1.3 (M) 6.0 (F)	Decreased weight gain	Lee et al., 1985
2,4-DNT	rat/CD	M/F	16/16	0.422 (M) 0.267 (F)	diet	0.2% in feed for 13 weeks	93 (M) 108 (F)	16.9 (M) 16.9 (F)	Weight loss; reticulocytosis; organ weight changes; abnormal pigments in spleen; decreased spermatogenesis; demyelination (males)	Lee et al., 1985
2,4-DNT	rat/CD	M/F	16/16	0.279 (M) 0.241 (F)	diet	0.7% in feed for 13 weeks	266 (M) 145 (F)	42.2 (M) 21.9 (F)	Same as 0.2% plus increased mortality; anemia	Lee et al., 1985
2,4-DNT	rat/CD	M/F	38/38	0.65 (M) 0.39 (F)	diet	0.0015% in feed for 24 months	0.57 (M) 0.71 (F)	0.12 (M) 0.13 (F)	Mild hepatocellular alterations	Lee et al., 1985

TABLE 9-1 (cont.)

Compound	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Dosage/Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Response	Reference
2,4-DNT	rat/CD	M/F	38/38	0.61 (M) 0.37 (F)	diet	0.01% in feed for 24 months	3.92 (M) 5.14 (F)	0.81 (M) 0.90 (F)	Decreased weight gain; anemia; mild hepatocellular alterations; testicular atrophy	Lee et al., 1985
2,4-DNT	rat/CD	M/F	38/38	0.47 (M) 0.29 (F)	diet	0.07% in feed for 24 months	34.5 (M) 45.3 (F)	6.5 (M) 7.3 (F)	Decreased weight gain; anemia; increased mortality; altered organ weights; marked hepatocellular alterations; neoplastic nodules; testicular atrophy; excessive pigmentation of spleen; hepatocellular carcinoma; decreased spermatogenesis; increased incidence of subcutaneous tumors	Lee et al., 1985
2,4-DNT	mouse/ CD-1	M/F	16/16	0.3 (M/F)	diet	0.07% in feed for 13 weeks	47 (M) 52 (F)	3.5 (M) 3.9 (F)	Decreased weight gain; (males)	Hong et al., 1985
2,4-DNT	mouse/ CD-1	M/F	16/16	0.03 (M/F)	diet	0.2% in feed for 13 weeks	137 (M) 147 (F)	10.3 (M) 11.1 (F)	Decreased weight gain; (males); hepatocellular dysplasia (males)	Hong et al., 1985
2,4-DNT	mouse/ CD-1	M/F	16/16	0.03 (M/F)	diet	0.7% in feed for 13 weeks	413 (M) 468 (F)	31.3 (M) 35.3 (F)	Weight loss; anemia; testicular degeneration; hepatocellular dysplasia; pigment deposition in liver	Hong et al., 1985
2,4-DNT	mouse/ CD-1	M/F	38/38	0.042 (M)	diet	0.01% in feed for 24 months	14	1.2	Decreased weight gain (males); abnormal pigmentation; renal tumors (male); hepatocellular dysplasia (male)	Hong et al., 1985
2,4-DNT	mouse/ CD-1	M/F	38/38	0.038 (M)	diet	0.07% in feed for 24 months	95	7.7	Decreased weight gain; (males); abnormal pigmentation; renal tumors (male); hepatocellular dysplasia (male); testicular atrophy; decreased spermatogenesis	Hong et al., 1985

TABLE 9-1 (cont.)

Compound	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Dosage/Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Response	Reference
2,4-DNT	mouse/ CD-1	M/F	38/38	0.03 (M/F)	diet	0.7% in feed for 24 months	898	68	Increased mortality; decreased weight gain; toxic anemia; reticulocytosis; altered organ weights; abnormal pigmentation; renal tumors (male); hepatocellular dysplasia; testicular or ovarian atrophy	Hong et al., 1985
2,4-DNT	rat/ Wistar	M	20	0.18	diet	0.5% in feed for 6 months	392[b]	54	Weight loss; increased mortality; altered organ weights; increased methemoglobin; increased level of various serum components and enzymes	Kozuka et al., 1979
2,6-DNT	dog/ beagle	M/F	NR	12.7	lactose/ gelatin capsule	4, 20 or 100 mg/kg/day for 90 days	4 20 100	2.3 11.3 56.6	Decreased muscular coordination; decreased appetite; weight loss increased mortality (high dose); methemoglobinemia; anemia; reticulocytosis; extramedullary hematopoiesis in spleen, liver, gliosis and demyelination in brain; testicular atrophy with aspermatogenesis	Ellis et al., 1976[c]
2,6-DNT	rat/CD	M/F	NR	0.35	diet	0.01, 0.05 or 0.25% in feed for 90 days	5[b] 25 125	0.85 4.3 21.4	Same as for dogs	Ellis et al., 1976[c]
2,6-DNT	mouse/ CD-1	M/F	NR	0.03 (M/F)	diet	0.01, 0.05 or 0.25% in feed for 90 days	13 65 325	0.98 4.9 2.5	Same as for dogs except that muscular coordination was not affected	Ellis et al., 1976
t-DNT	rat/ Fischer 344	M/F	130/130	0.35 (M) 0.24 (F)	diet	3.5 mg/kg/day in feed for 104 weeks	3.388 (M) 3.379 (F)	0.58 (M) 0.51 (F)	Decreased weight gain; increased liver and kidney weights, hepatic neoplasms; hepatotoxicity (necrosis); various histologic tissue alterations; enhanced chronic interstitial nephritis	CIIT, 1982

TABLE 9-1 (cont.)

Compound	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Dosage/Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Response	Reference
t-DNT	rat/ Fischer 344	M/F	130/130	0.29 (M) 0.19 (F)	diet	14.0 mg/kg/day in feed for 104 weeks	13.451 (M) 13.633 (F)	2.16 (M) 1.90 (F)	Decreased weight gain; increased weights of liver, kidney and brain; hepatotoxicity; hepatocellular carcinoma; hepatic neoplastic nodules; degenerative changes in adrenal glands; reduced survival rate (males)	CIIT, 1982
t-DNT	rat/ Fischer 344	M/F	130/130	0.25 (M) 0.17 (F)	diet	35.0 mg/kg/day in feed for 55 weeks	35.0	5.3 (M) 4.7 (F)	Decreased weight gain; anemia; increased SGPT, BUN; increased organ weights; hepatotoxicity; hepatocellular carcinoma; hepatic neoplastic nodules; various histologic tissue alterations; reduced survival rate (males)	CIIT, 1982

[a] Assumes that rats and mice consume the equivalent of 0.05 and 0.13, respectively, of their body weights as food/day.

[b] This dose was estimated from the reported experimental data.

[c] This report is an abstract only. No details were given as to the effects of dose level on toxicity nor were differences between males and females discussed.

NR = Not reported

secondary to tumorigenesis, has also been observed. In addition, reduced fetal survival accompanied by severe maternal toxicity was observed.

Data used to calculate CSs and RQs are presented in Table 9-2. The MEDs were calculated by multiplying the equivalent human dose in mg/kg/day by 70 kg to express the MED in mg/day. Because chronic studies that examined the same endpoints as the subchronic studies were available for 2,4-DNT, it is not necessary to consider the subchronic studies for the derivation of the RQ based on chronic toxicity for this isomer. As seen from Table 9-1, the most serious effect of 2,4-DNT was reduced survival, which warrants an RV_e of 10. The lowest equivalent dose at which reduced survival occurred is 6.5 mg/kg/day in the chronic rat study by Lee et al. (1985). The MED, therefore, is 455 mg/day, which corresponds to an RV_d of 1.5. Multiplying the RV_d by the RV_e results in a CS of 15, which corresponds to an RQ of 1000. It is not necessary to calculate CSs for the less serious effects that occurred at equivalent human doses of $\geq$6.5 mg/kg/day.

The next most serious effect of 2,4-DNT in chronic studies is the neurotoxicity, neuromuscular incoordination and paralysis of dogs, which warrants an RV_e of 9. These effects occurred at equivalent human doses $\geq$0.85 mg/kg/day in the study by Ellis et al. (1985). The MED is 59.5 mg/day, which corresponds to an RV_d of 2.8. Multiplying the RV_d by the RV_e results in a CS of 25.2, which corresponds to an RQ of 100. Again, it is not necessary to calculate CSs for the less serious effects that occurred at equivalent human doses $\geq$0.85 mg/kg/day. Similarly, it is not necessary to calculate CSs for the less severe effect of testicular atrophy that occurred at an equivalent human dose of 0.81 mg/kg/day because, while the RV_d is essentially the same as for 0.85 mg/kg/day, the RV_e of 4 for atrophy is much lower than that for severe neurotoxic effects.

TABLE 9-2

Oral Composite Scores for the DNTs

Compound	Species	Animal Dose (mg/kg/day)	Chronic Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ	Reference
2,4-DNT	rat	34.5	455	1.5	reduced survival	10	15	1000	Lee et al., 1985
2,4-DNT	dog	1.5	59.5	2.8	neurotoxicity, neuromuscular incoordination, paralysis	9	25.2	100	Ellis et al., 1985
t-DNT	rat	3.379	35.7	3.2	enhanced chronic interstitial nephritis	6	19	1000	CIIT, 1982
t-DNT	rat	2.16	151.2	2.2	reduced survival	10	22	100	CIIT, 1982

At equivalent human doses $\geq$0.12 mg/kg/day, there were mild hepatocellular alterations in rats after 1 year in the chronic study by Lee et al. (1985). After 2 years, however, some rats treated at this level had hepatocellular neoplastic nodules. Hepatocellular alterations, therefore, may be considered preneoplastic lesions, which are inappropriate for consideration in determining an RQ based on chronic toxicity. As seen from Table 9-2, the highest CS resulted from the data for neurotoxicity in dogs in the study by Ellis et al. (1985); the resultant RQ of 100 is recommended for 2,4-DNT (Table 9-3). This assessment disagrees with the CS recommended by U.S. EPA (1983a), which derived a CS of 32 based on the effects in dogs receiving 5 mg/kg/day for 90 days. These data, however, were obtained from an abstract (Ellis et al., 1976) that provided very little detail. Ellis et al. (1985) updated this report of the 90-day study and also reported the results of the chronic study. The current determination more appropriately uses the chronic data. Nevertheless, the RQ remains 100.

The only available study on 2,6-DNT was reported in an abstract by Ellis et al. (1976) (see Table 9-1). The effects of 2,6-DNT in dogs, rats and mice in this subchronic study were reported to be similar to those observed for 2,4-DNT by Ellis et al. (1976, 1985), Lee et al. (1985) and Hong et al. (1985). Although U.S. EPA (1983b) did derive an RQ for 2,6-DNT based on the information in the abstract, unfortunately, the abstract did not report dose-response relationships, precluding a confident determination of the MED for the various effects. Because, however, the effects were similar to those 2,4-DNT, it is reasonable to assign an RQ of 100 to 2,6-DNT by analogy to 2,4-DNT (Table 9-4).

TABLE 9-3

2,4-DNT

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	59.5 mg/day
Effect:	neurotoxicity, neuromuscular incoordination, paralysis
Reference:	Ellis et al., 1985
RV_d:	2.8
RV_e:	9
Composite Score:	25.2
RQ:	100

---

*Equivalent human dose

TABLE 9-4

2-6-DNT

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose[a]:	59.5 mg/day[b]
Effect:	neurotoxicity, neuromuscular incoordination, paralysis
Reference:	by analogy to 2,4-DNT
RV_d:	2.8
RV_e:	9
Composite Score:	25.2
RQ:	100

---

[a] Equivalent human dose

[b] The study was performed with 2,4-DNT

For technical DNT, lifetime feeding studies in rats resulted in hepatocellular neoplasms (CIIT, 1982) (see Table 9-1); therefore, it is likely that the hepatoxicity is related to the hepatic tumorigenicity, and it is not appropriate to derive an RQ for hepatotoxicity.

Of the non-neoplastic lesions, the most severe was the enhancement of chronic interstitial nephritis, which warrants an RV_e of 6. This occurred at equivalent human doses $\geq$0.51 mg/kg/day. Multiplying this dose by 70 kg results in an MED of 35.7 mg/day, which corresponds to an RV_d of 3.2. This CS is 22.4, which corresponds to an RQ of 1000.

There was reduced survival (RV_e=10) at equivalent human doses $\geq$2.16 mg/kg/day or an MED of 151.2 mg/day, which corresponds to an RV_d of 2.2. The CS is 22 and the RQ is 100, which is recommended for technical grade DNT (Table 9-5).

Data regarding the subchronic or chronic toxicity of 2,3-, 2,5- and 3,4-DNT could not be located in the available literature as cited in the Appendix; therefore, data are not sufficient to derive an RQ (Table 9-6).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

The relevant bioassays regarding the carcinogenicity of DNT were discussed in Section 5.1. and are summarized in Tables 5-1 through 5-4. As indicated in Table 5-1, dietary administration of 80 and 200 ppm 2,4-DNT for 78 weeks to rats resulted in a dose-related increase in subcutaneous fibromas in males and a significantly increased incidence of mammary gland fibroadenomas in females (NCI, 1978). In light of recent evidence concerning the initiating and promoting properties of 2,4- and 2,6-DNT (Popp and Leonard, 1982; Leonard et al., 1983), it is not possible to tell whether this weak tumorigenic response was due to the 2,4-DNT (purity >95%) or to an impurity, possibly 2,6-DNT.

TABLE 9-5

Technical Grade DNT

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	151.2 mg/day
Effect:	reduced survival rate
Reference:	CIIT, 1982
RV_d:	2.2
RV_e:	10
Composite Score:	22
RQ:	100

---

*Equivalent human dose

TABLE 9-6

2,3-, 2,5- and 3,4-DNT

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:

Dose:

Effect:

Reference:

RV_d:

RV_e:

Composite Score:

RQ: Data are not sufficient to derive an RQ

Ellis et al. (1979) exposed male and female rats to 15, 100 and 700 ppm 2,4-DNT in the diet for 2 years and reported an increased incidence of hepatocellular carcinomas in the high-dose group, although a statistically significant increase was observed only in females. An increased incidence of mammary gland benign tumors was also noted in high-dose females (see Table 5-2). This study also reported a significant increase in the number of male mice with benign and malignant renal tumors after administration of 700 ppm 2,4-DNT for 2 years (see Table 5-3). As with the NCI (1978) study, the presence of impurities in the test compound may have affected the observed results. Furthermore, analysis of the 2,4-DNT used in this study resulted in the detection of ~2% 2,6-DNT, which may have been sufficient to influence the rate of tumor formation.

The bioassay conducted by CIIT (1982) used technical grade DNT and made no attempt to distinguish which isomers were responsible for the observed effects. Male rats receiving a dose equivalent to 35 mg/kg/day had a 100% incidence of hepatocellular carcinomas after 52 weeks of treatment; females had a 50% incidence at the same time point. Lower doses produced similar lesions at later time points in both sexes (see Table 5-4). Other affected organs included the lung, testes, mammary gland and skin.

In vivo assays designed to test the initiating and promoting potential of all of the DNT isomers demonstrated that while both the 2,6- and 2,4-isomers were somewhat effective in promoting diethylnitrosamine-induced hepatocarcinogenicity, only 2,6-DNT had detectable initiating activity (Popp and Leonard, 1982; Leonard et al., 1983). Similar results were reported by Slaga et al. (1985) in a skin tumor initiation/promotion bioassay. In a very brief summary, Popp and Leonard (1982) reported that purified 2,6-DNT was able to induce a 100% incidence of hepatocellular carcinomas after 1 year while treatment with 2,4-DNT produced no tumors.

There is, therefore, sufficient evidence that DNTs as a class are carcinogenic to animals. Recent studies (Popp and Leonard, 1982; Leonard et al., 1983) strongly suggest that 2,6-DNT is the isomer primarily responsible for the carcinogenic activity observed with technical grade DNT, while 2,4-DNT may contribute more to the promoting phase of the carcinogenic response. Nevertheless, metabolic studies (see Section 4.3.) have indicated that 2,4-DNT can be metabolized to 2,4-diaminotoluene, a known carcinogen (NCI, 1979). No data were available indicating that any of the other DNT isomers are carcinogenic.

No case reports or epidemiological studies relating technical grade DNT or any DNT isomers to cancer in humans have been located. Therefore, technical grade DNT and 2,4- and 2,6-DNT are most appropriately classified as CAG Group B2 and Group C chemicals, respectively (U.S. EPA, 1984b).

A potency (F) factor for carcinogenicity can be derived for 2,4-DNT from the dose-response data for liver and mammary gland tumors in female rats and for kidney tumors in male mice (Ellis et al., 1979). The data for these calculations using the rat study are presented in Table 9-7. The transformed doses were calculated from reported intake levels (0, 0.706, 5.14 and 45.3 mg/kg/day) and were adjusted for the differences in body weight for each treatment group by multiplying the dose by the cube root of the animal to human (70 kg) body weight ratio. Thus, the dose was adjusted to extrapolate from rats to humans before calculating the $1/ED_{10}$. No time-to-tumor correction was necessary. The F factor of 3.99 $(mg/kg/day)^{-1}$ was calculated using the computerized multi-stage model developed by Howe and Crump (1982). Table 9-8 summarizes the data used to calculate the F factor for mice in the Ellis et al. (1979) study. A procedure similar to that detailed above was used except that an unadjusted $1/ED_{10}$ of $1.41x10^{-1}$

TABLE 9-7

Derivation of Potency Factor (F)
Agent: 2,4-DNT

Reference: Ellis et al., 1979

Exposure route: Oral

Species: Rat

Strain: Sprague-Dawley

Sex: Female

Vehicle or physical state: Diet

Body weight: 0.425 kg (control, low dose); 0.410 kg (mid dose); 0.325 kg (high dose) (reported)

Duration of treatment: 24 months

Duration of study: 24 months

Lifespan of animal: 24 months

Target organ: Liver; mammary gland

Tumor type: Hepatocellular carcinoma, neoplastic nodule (liver); adenoma, fibroadenoma, fibroma, adenocarcinoma-carcinoma (mammary gland)

Experimental doses/exposure: 0, 15, 100, 700 ppm or 0, 0.706, 0.514, 45.3 mg/kg/day

Transformed doses (mg/kg/day): 0, 0.129, 0.927, 7.557

Tumor incidence: 11/23, 12/35, 17/27, 34/35

$1/ED_{10}$ Unadjusted: not calculated (see text)

F factor: 3.9875111 $(mg/kg/day)^{-1}$

TABLE 9-8

Derivation of Potency Factor (F)
Agent: 2,4-DNT

Reference: Ellis et al., 1979

Exposure route: Oral

Species: Mouse

Strain: Swiss

Sex: Male

Vehicle or physical state: Diet

Body weight: 0.04 kg (reported)

Duration of treatment: 24 months

Duration of study: 24 months

Lifespan of animal: 24 months

Target organ: Kidney

Tumor type: Cystic papillary adenoma, cystic papillary carcinoma, solid renal cell carcinoma

Experimental doses/exposure: 0, 100, 700 ppm

Transformed doses (mg/kg/day): 0, 13.3, 96.9

Tumor incidence: 0/20, 4/21, 16/17

$1/ED_{10}$ Unadjusted: 1.413589×10^{-1} $(mg/kg/day)^{-1}$

F factor: 1.7034753 $(mg/kg/day)^{-1}$

$(mg/kg/day)^{-1}$ was calculated because the average body weights for all treatment groups were similar. The transformed doses in Table 9-10 were as reported in the study. The unadjusted $1/ED_{10}$ was multiplied by the cube root of the ratio of human weight (70 kg) to the average weight of the male mice (0.04 kg) to arrive at a potency factor of 1.70 $(mg/kg/day)^{-1}$.

Potency factors were also calculated for technical grade DNT from data regarding increased incidences of hepatocellular carcinomas and hepatic neoplastic nodules in male and female rats (CIIT, 1982). Tables 9-9 and 9-10 include the data necessary for these calculations. Since the estimated body weights for all treatment groups in both sexes were different, an adjustment was made before calculating the ED_{10} to extrapolate each dose from rats to humans by multiplying by the cube root of the ratio of the reported rat body weight (see Tables 9-9 and 9-10) to the reference human body weight (70 kg). Since this correction was made and no other corrections were needed, the $1/ED_{10}$ or F factor for humans of 2.58 and 2.83 $(mg/kg/day)^{-1}$ were calculated directly using the multistage model.

Since the F factors fall between 1 and 10, 2,4-, 2,6- and technical grade DNT are placed in Potency Group 3. A CAG Group B2 or Group C and a Potency Group 3 chemical has a MEDIUM (2,4-DNT) or LOW (2,6-DNT) hazard ranking under CERCLA.

TABLE 9-9

Derivation of Potency Factor (F)
Agent: Technical DNT

Reference: CIIT, 1982

Exposure route: Oral

Species: Rat

Strain: Fischer 344

Sex: Male

Vehicle or physical state: Diet

Body weight: 0.37 kg (control); 0.35 kg (low dose); 0.29 kg (high dose) (reported)

Duration of treatment: 104 weeks

Duration of study: 104 weeks

Lifespan of animal: 104 weeks

Target organ: Liver

Tumor type: Hepatocellular carcinoma, neoplastic nodule

Experimental doses/exposure: 0, 3.388, 13.451 mg/kg/day

Transformed doses (mg/kg/day): 0, 0.579, 2.160

Tumor incidence: 10/61, 19/70, 23/23

$1/ED_{10}$ Unadjusted: Not calculated (see text)

F factor: 2.5800193 $(mg/kg/day)^{-1}$

TABLE 9-10

Derivation of Potency Factor (F)
Agent: Technical DNT

Reference: CIIT, 1982

Exposure route: Oral

Species: Rat

Strain: Fischer 344

Sex: Female

Vehicle or physical state: Diet

Body weight: 0.26 kg (control); 0.24 kg (low dose); 0.19 kg (high dose) (reported)

Duration of treatment: 104 weeks

Duration of study: 104 weeks

Lifespan of animal: 104 weeks

Target organ: Liver

Tumor type: Hepatocellular carcinoma, neoplastic nodule

Experimental doses/exposure: 0, 3.379, 13.633 mg/kg/day

Transformed doses (mg/kg/day): 0, 0.51, 1.902

Tumor incidence: 5/57, 12/61, 66/68

$1/ED_{10}$ Unadjusted: Not calculated (see text)

F factor: 2.8338326 $(mg/kg/day)^{-1}$

DINiTROTOLUENES

10. REFERENCES

Abernethy, D.J. and D.B. Couch. 1982. Cytotoxicity and mutagenicity of dinitrotoluenes in Chinese hamster ovary cells. Mutat. Res. 103(1): 53-59.

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values for Substances in Workroom Air, 4th ed. with supplements through 1984. Cincinnati, OH. p. 153-154.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985. TLVs: Threshold Limit Values for Chemical Substances in the Work Environment Adopted by ACGIH for 1985-1986. Cincinnati, OH. p. 17.

Adema, D.M.M., J. Kuiper, A.O. Hanstveit and H.H. Canton. 1983. Consecutive system of tests for assessment of the effects of chemical agents in the aquatic environment. In: Pestic. Chem.: Human Welfare Environ. Proc. 5th Int. Congr. Pestic. Chem. Meeting Date 1982, J. Miyamoto and P.C. Kearney, Ed. 3: 537-544.

Ahrenholz, S.H. 1980. Health hazard evaluation determination report No. HE 79-113-728, Olin Chemical Company, Brandenburg, KY. Report NIOSH HE-79-113. NTIS PB81-167819. (CA 95:155707w)

Albright, L.F. 1981. Nitration. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 15, 3rd ed., M. Grayson and D. Eckroth, Ed. John Wiley and Sons, Inc., New York. p. 846.

Aldrich. 1984. 1984-1985 Aldrich Catalog/Handbook of Fine Chemicals. Aldrich Chemical Co., Milwaukee, WI. p. 468.

Back, K.C., A.A. Thomas and J.D. MacEwen. n.d. Reclassification of materials listed as transportation health hazards. 6570th Aerospace Medical Research Laboratory, Report No. TSA-20-72-3, Wright-Patterson Air Force Base, OH. (Cited in Rickert et al., 1984)

Bailey, H.C. 1982. Development and testing of a laboratory model ecosystem for use in evaluating biological effects and chemical fate of pollutants. ASTM Spec. Tech. Publ. 766: 221-233.

Bailey, H.C. and R.J. Spanggord. 1983. The relationship between the toxicity and structure of nitroaromatic chemicals. ASTM Spec. Tech. Publ. 802: 98-107.

Barth, E.F. and R.L. Bunch. 1979. Biodegradation and treatability of specific pollutants. U.S. EPA, Municipal Environmental Research Laboratory, Cincinnati, OH. EPA-600/9-79-034.

Bermudez, E., D. Tillery and B.E. Butterworth. 1979. The effect of 2,4-diaminotoluene and isomers of dinitrotoluene on unscheduled DNA synthesis in primary rat hepatocytes. Environ. Mutagen. 1(4): 391-398.

Bond, J.A. and D.E. Rickert. 1981. Metabolism of carbon-14-labeled 2,4 dinitrotoluene by freshly isolated Fischer 344 rat primary hepatocytes. Drug Metab. Dispos. 9(1): 10-14.

Bond, J.A., M.A. Medinsky, J.G. Dent and D.E. Rickert. 1981. Sex-dependent metabolism and biliary excretion of [2,4-^{14}C]dinitrotoluene in isolated perfused rat livers. J. Pharmacol. Exp. Ther. 219(3): 598-603.

Borzelleca, J.F. and R.A. Carchman. 1982. Effects of selected organic drinking water contaminants on male reproduction. EPA-600/1 82- 009. NTIS PB82-259847. 149 p.

Bringmann, G. and R. Kuehn. 1977. Results of the damaging effect of water pollutants on *Daphnia magna*. Z. Wasser Abwasser Forsch. 10(5): 161-166.

Bringmann, G. and R. Kuehn. 1978. Testing of substances for their toxicity threshold: Model organisms *Microcystis* (*Diplocystis*) *aeruginosa* and *Scenedesmus quadricauda*. Mitt. Internat. Verein. Limnol. 21: 275-284.

Bringmann, G. and R. Kuehn. 1980. Comparison of the toxicity thresholds of water pollutants to bacteria algae, and protozoa in the cell multiplication inhibition test. Water Res. 14(3): 231-241.

Bringmann, G. and R. Kuehn. 1981. Comparison of the effect of harmful substances on flagellates and ciliates as well as on bacteriovorous and saprozoic protozoans. GWF, GasWasserfach: Wasser/Abwasser. 122(7): 308-313.

Bringmann, G. and R. Kuehn. 1982. Results of toxic action of water pollutants on *Daphnia magna* Straus tested by an improved standardized procedure. Z. Wasser Abwasser Forsch. 15(1): 1-6.

Buccafusco, R.J., S.J. Ells and G.A. LeBlanc. 1981. Acute toxicity of priority pollutants to bluegill (Lepomis macrochirus). Bull. Environ. Contam. Toxicol. 26(4): 446-452.

Chiu, C.W., L.H. Lee, C.Y. Wang and G.T. Bryan. 1978. Mutagenicity of some commercially available nitro compounds for Salmonella typhimurium. Mutat. Res. 58(1): 11-22.

CIIT (Chemical Industry Institute of Toxicology). 1982. 104-Week chronic toxicity study in rats: Dinitrotoluene. Final Report. CIIT Docket #12362.

Clayton, C.C. and C.A. Baumann. 1944. Some effects of diet on the resistance of mice toward 2,4-dinitrotoluene. Arch. Biochem. 5: 115. (Cited in Rickert et al., 1984)

Clayton, C.C. and C.A. Baumann. 1948. Effect of fat and calories on the resistance of mice toward 2,4-dinitrotoluene. Arch. Biochem. 16: 415. (Cited in Rickert et al., 1984)

Couch, D.B., P.F. Allen and D.J. Abernethy. 1981. The mutagenicity of dinitrotoluenes in Salmonella typhimurium. Mutat. Res. 90(4): 373-384.

Davis, E.M., H.E. Murray, J.G. Liehr and E.L. Powers. 1981. Basic microbiol degradation rates and chemical by-products of selected organic compounds. Water Res. 15(9): 1125-1127.

Davis, E.M., J.E. Turley, D.M. Casserly and R.K. Guthrie. 1983. Partitioning of selected organic pollutants in aquatic ecosystems. In: 5th Meet. Symp. Pap. Int. Biodeterior, 1981. p. 176-184.

Decad, G.M., M.E. Graichen and J.G. Dent. 1982. Hepatic microsomal metabolism and covalent binding of 2,4-dinitrotoluene. Toxicol. Appl. Pharmacol. 62(2): 325-334.

Dent, J.G., S.R. Schnell and D. Guest. 1982. Metabolism of 2,4-dinitrotoluene by rat hepatic microsomes and cecal flora. Adv. Exp. Med. Biol. 136A: 431-436.

DeWalle, F.B. and E.S.K. Chian. 1978. Presence of trace organics in the Delaware River and their discharge by municipal and industrial sources. In: Proc. Ind. Waste Conf. 32: 908-919.

Dunlap, K.L. 1981. Nitrobenzene and nitrotoluenes. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 15, 3rd ed., M. Grayson and D. Eckroth, Ed. John Wiley and Sons, Inc., New York. p. 925-926, 930-931.

Durkin, P.R. 1985. Reference Values for Risk Assessment. Prepared by Syracuse Research Corporation, Syracuse, NY, under Contract No. 68-02-3228 for Environmental Criteria and Assessment Office, U.S. EPA, Cincinnati, OH.

Ellis, H.V., III, J.V. Dilley and C.C. Lee. 1976. Subacute toxicity of 2,4-dinitrotoluene and 2,6-dinitrotoluene. Toxicol. Appl. Pharmacol. 37(1): 116-117.

Ellis, H.V., III, J.H. Hagensen, J.R. Hodgson, J.L. Minor and C.B. Hong. 1979. Mammalian toxicity of munition compounds. Phase III. Effects of lifetime exposure. Part I. 2,4-Dinitrotoluene. Report Order No. AD-A077692. 281 p.

Ellis, H.V., C.B. Hong and C.C. Lee. 1980. Mammalian toxicity of munition compounds. Summary of toxicity of nitrotoluenes, Progress Report No. 11, Midwest Research Institute Project 3900-B. (Cited in Rickert et al., 1984)

Ellis, H.V., III, C.B. Hong, C.C. Lee, J.C. Dacre and J.P. Glennon. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dogs. J. Am. Coll. Toxicol. 4(4): 233-242.

Ewing, B.B., E.S.K. Chian, J.C. Cook, C.A. Evans, P.K. Hopke and E.G. Perkins. 1977. Monitoring to detect previously unrecognized pollutants in surface waters. Appendix: Organic analysis data. U.S. EPA, Washington, DC. EPA-560-6-77-015.

Fannick, N. 1981. Health hazard evaluation determination report No. HHE 77-99-726, Du Pont, Chambers Works, Deepwater, NJ. NTIS PB81-167843. 10 p.

Floret, F. 1929. Medical opinions on industrial poisonings. Centr. Gewerbehyg. Unfallverhut. 16: 280. (Cited in U.S. EPA, 1980a)

Guest, D., S.R. Schnell, D.E. Rickert and J.G. Dent. 1982. Metabolism of 2,4-dinitrotoluene by intestinal microorganisms from rat, mouse, and man. Toxicol. Appl. Pharmacol. 64(1): 160-168.

Hallas, L.E. and M. Alexander. 1983. Microbial transformation of nitroaromatic compounds in sewage effluent. Appl. Environ. Microbiol. 45(4): 1234-1241.

Hamill, P.V.V., E. Steinberger, R.J. Levine, L.J. Rodriguez-Rigan, S. Lemeshow and J.S. Avrunin. 1982. The epidemiologic assessment of male reproductive hazard from occupational exposure to TDA and DNT. J. Occ. Med. 24(12): 985-993.

Hartley, W.R. 1981. Evaluation of selected subacute effects of the nitrotoluene group of munitions compounds on fish and potential use in aquatic toxicity evaluation. NTIS AD-A101829. 223 p.

Hashimoto, A., H. Sakine, T. Kojima, E. Yamagami, S. Tateishi and T. Akiyama. 1982. Sources and behavior of dinitrotoluene isomers in seawater. Water Res. 16(6): 891-897.

Hawley, G.G., Ed. 1981. The Condensed Chemical Dictionary, 10th ed. Von Nostrand Reinhold Co., New York. p. 375.

Heitmuller, P.T., T.A. Hollister and P.R. Parrish. 1981. Acute toxicity of 54 industrial chemicals to sheepshead minnows (Cyprinodon variegatus). Bull. Environ. Contam. Toxicol. 27(5): 596-604.

Hodgson, J.R., M.A. Kowalski, J.P. Glennon, J.C. Dacre and C.C. Lee. 1976. Mutagenicity studies on 2,4-dinitrotoluene. Mutat. Res. 38: 387.

Hodgson, J.R., S.W. Hwang, J.C. Dacre and C.C. Lee. 1977. Comparative absorption, distribution, excretion and metabolism of 2,4,6-trinitrotoluene (TNT) and isomers of dinitrotoluene (DNT) in rats. Fed. Proc. 36(1): 996.

Hoff, M.C. 1983. Toluene. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 23, 3rd ed., M. Grayson and D. Eckroth, Ed. John Wiley and Sons, Inc., New York. p. 265-266.

Hong, C.B., H.V. Ellis, III, C.C. Lee, H. Sprinz, J.C. Dacre and J.P. Glennon. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part III. CD-1 mice. J. Am. Coll. Toxicol. 4(4): 257-269.

Howe, R.B. and K.S. Crump. 1982. GLOBAL 82. A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses. Prepared for Office of Carcinogen Standards, OSHA, U.S. Dept. of Labor under Contract No. 4145C25C3.

Isbister, J.D., R.C. Doyle and J.F. Kitchens. 1980. Engineering and development support of general decon technology for the DARCOM installation restoration program. Task 6. Adapter/mutant biological treatment. Phase I. Literature review. Gov. Rep. Announce. Index (U.S.) 1982. 82(11): 2225. Report DRXTH-IS-CR 80132. NTIS AD-A110389.

Jaffe, L.S., R.W. Tew, D.W. Burrows and J.C. Dacre. 1973. Mammalian toxicity and toxicity to aquatic organisms of TNT, DNT and other munitions manufacturing waste constituents of pint water. Literature evaluation. NTIS AD777903/6GA. 80 p.

Kenaga, E.E. 1980. Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. Ecotox. Environ. Saf. 4: 26-38.

Kool, H.J., C.F. van Kretjl and B.C.J. Zoeteman. 1982. Toxicology assessment of organic compounds in drinking water. Crit. Rev. Environ. Contam. 12: 307-357.

Kozuka, H., M. Mori, K. Katayama, et al. 1978. Studies on the metabolism and toxicity of dinitrotoluenes - Metabolism of dinitrotoluenes by _Rhodotorula glutinis_ and rat liver homogenate. Eisei Kagaku. 24(5): 252-259.

Kozuka, H., M.A. Mori and Y. Naruse. 1979. Metabolism and toxicity of dinitrotoluenes: Toxicological study of 2,4-dinitrotoluene in rats in long-term feeding. J. Toxicol. Sci. 4(3): 221-228.

LeBlanc, G.A. 1980. Acute toxicity of priority pollutants to water flea (_Daphnia magna_). Bull. Environ. Contam. Toxicol. 24(5): 684-691.

LeBlanc, G.A. 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. Environ. Toxicol. Chem. 3(1): 47-60.

Lee, C.C., H.V. Ellis, III, J.R. Hodgson, et al. 1976. Mammalian toxicity of munition compounds. Phase II. Effects of multiple doses. Part III: 2,6-Dinitrotoluene. Report No. 4, Midwest Research Institute Project No. 3900-B. (Cited in Rickert et al., 1984)

Lee, C.C., H.V. Ellis, III, J.J. Kowalski, et al. 1978. Mammalian toxicity of munition compounds. Phase II. Effects of multiple doses. Part II. 2,4-Dinitrotoluene. Midwest Research Institute, Kansas City, MO. NTIS AD A061715. (Cited in TSCA-ITC, 1981; Rickert et al., 1984; U.S. EPA, 1980a)

Lee, C.C., C.B. Hong, H.V. Ellis, III, J.C. Dacre and J.P. Glennon. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part II. CD rats. J. Am. Coll. Toxicol. 4(4): 243-256.

Leonard, T.B., O. Lyght and J.A. Popp. 1983. Dinitrotoluene structure-dependent initiation of hepatocytes _in vivo_. Carcinogenesis (London). 4(8): 1059-1061.

Lewin, L. 1921. Poisoning from trinitrotoluene. Arch. Exp. Pathol. Pharmakol. 89: 340. (Cited in U.S. EPA, 1980a)

Liu, D.H.W., R.J. Spanggord, H.C. Bailey, H.S. Javitz and D.C.L. Jones. 1983a. Toxicity of TNT wastewaters to aquatic organisms. Volume 2. Acute toxicity of condensate wastewater and 2,4-dinitrotoluene. NTIS AD-A142145. 70 p. (CA 102:1516j)

Liu, D.H.W., H.C. Bailey and J.G. Pearson. 1983b. Toxicity of a complex munitions wastewater to aquatic organisms. ASTM Spec. Tech. Publ. 802: 135-150.

Long, R.M. and D.E. Rickert. 1982. Metabolism and excretion of 2,6-dinitro[^{14}C]toluene _in vivo_ and in isolated perfused rat livers. Drug Metab. Dispos. 10(5): 455-458.

Lyman, W.J., W.F. Reehl and D.H. Rosenblatt. 1982. Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co., New York. p. 4-9, 15-16.

MacEwen, J.D. and E.H. Vernot. 1972. Toxic Hazards Research Unit Annual Technical Report: 1972. NTIS AD-755358. 151 p. (Cited in NIOSH, 1977)

Maksimov, Y.Y. 1968. Vapor pressures of aromatic nitrocompounds at various temperatures. Zh. Fiz. Khim. 42(11): 2921-2925. (Russ.) (CA 80:61315y)

Matsui, S., T. Murakami, T. Sasaki, Y. Hirose and Y. Iguma. 1975. Activated sludge degradability of organic substances in the wastewater of the Kashima Petroleum and Petrochemical Industrial Complex in Japan Prog. Water Technol. 7(2-3-4): 645-659.

McCormick, N.G., F.E. Feeherry and H.S. Levinson. 1976. Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. Appl. Environ. Microbiol. 31(6): 949-958.

McCormick, N.G., J.H. Cornell and A.M. Kaplan. 1978. Identification of biotransformation products from 2,4-dinitrotoluene. Appl. Environ. Microbiol. 35: 945-948.

McGee, L.C., A. McCausland, C.A. Plume, et al. 1942. Metabolic disturbances in workers exposed to dinitrotoluenes. Am. J. Digest. Diseases. 9: 329-331. (Cited in U.S. EPA, 1980a; TSCA ITC, 1981)

McGee, L.C., H.L. Reed, T.J. Nerein, et al. 1947. Metabolic disturbances in workers exposed to dinitrotoluene during World War II. Gastroenterology. 8: 293-295. (Cited in TSCA-ITC, 1981)

Medinsky, M.A. and J.G. Dent. 1983. Biliary excretion and enterohepatic circulation of 2,4-dinitrotoluene metabolites in Fischer-344 rats. Toxicol. Appl. Pharmacol. 68(3): 359-366.

Mirsalis, J.C. and B.E. Butterworth. 1982. Induction of unscheduled DNA synthesis in rat hepatocytes following *in vivo* treatment with dinitrotoluene. Carcinogenesis (Lond). 3(3): 241-246.

Mirsalis, J.C., T.E. Hamm, Jr., J.M. Sherrill and B.E. Butterworth. 1982. Role of gut flora in the genotoxicity of dinitrotoluene. Nature. 295(5847): P322-P323.

Mori, M., Y. Naruse and H. Kozuka. 1977. The metabolism and toxicity of dinitrotoluenes. The excretion and distribution of tritium-labeled-2,4-dinitrotoluene (3H-2,4-DNT) in the rat. Radioisotopes. 26(11): 780-783.

Mori, M., Y. Naruse and H. Kozuka. 1978. Studies on the metabolism and toxicity of dinitrotoluenes. Absorption and excretion of tritium-labeled 2,4-dinitrotoluene (3H-2,4-DNT) in rats. Radioisotopes. 27(12): 715-719.

Mori, M., Y. Naruse and H. Kozuka. 1980. Studies on the metabolism and toxicity of dinitrotoluenes. Changes of excretion, distribution and metabolism of 3H-2,4-dinitrotoluene (3H-2,4-DNT) in rats. Radioisotopes. 29(7): 338-340.

Mori, M., Y. Naruse and H. Kozuka. 1981. Identification of urinary metabolites of 2,4-dinitrotoluene (2,4-DNT) in rats. Chem. Pharm. Bull. 29(4): 1147-1150.

Mori, M.A., T. Miyahara, K. Taniguchi, K. Hasegawa, H.K.M. Miyagoshi and T. Nagayama. 1982. Mutagenicity of 2,4-dinitrotoluene and its metabolites in *Salmonella typhimurium*. Toxicol. Lett. (Amst). 13(1-2): 1-6.

Mori, M.A., T. Miyahara, Y. Hasegawa, Y. Kudo and H. Kozuka. 1984a. Metabolism of dinitrotoluene isomers by *Escherichia coli* isolated from human intestine. Chem. Pharm. Bull. (Tokyo). 32(10): 4070-4075.

Mori, M , T. Matsuhashi, T. Miyahara, S. Shibata, C. Izima and H. Kozuka. 1984b. Reduction of 2,4-dinitrotoluene by Wistar rat liver microsomal and cytosol fractions. Toxicol. Appl. Pharmacol. 76(1): 105-112.

Mori, M., Y. Kudo, T. Nunozawa, T. Miyahara and H. Kozuka. 1985. Intestinal metabolism of 2,4-dinitrotoluene in rats. Chem. Pharm. Bull. 33(1): 327-332.

NCI (National Cancer Institute). 1978. Bioassay of 2,4-dinitrotoluene for possible carcinogenicity. Carcinogenesis Tech. Rep. Ser. No. 54. Publ. No. 78-1360.

NCI (National Cancer Institute). 1979. Bioassay of 2,4-dinitrotoluene for possible carcinogenicity. NCI Carcinogenesis Tech. Rep. Ser. No. 162. 122 p.

NIOSH (National Institute for Occupational Safety and Health). 1977. Information profiles on potential occupational hazards: Nitrotoluenes. Prepared by Syracuse Research Corporation, Syracuse, NY under Contract No. 210-77-0120.

Parrish, F.W. 1977. Fungal transformation 2,4-dinitrotoluene and 2,4,6-trinitrotoluene. Appl. Environ. Microbiol. 34(2): 232-233.

Pella, P.A. 1977. Measurement of the vapor pressures of TNT, 2,4-DNT and EGDN. J. Chem. Thermodyn. 9: 301-305.

Perkins, R.G. 1919. A study of the munitions intoxications in France. U.S. Pub. Health Rep. 34: 2335-2374. (Cited in U.S. EPA, 1980a)

Piet, G.J. and C.F. Morra. 1983. Behaviour of organic micropollutants during underground passage. In: Artificial Groundwater Recharge (Water Resources Engineering Series), L. Huisman and T.N. Olsthorn, Ed. Pitman Publ., CITY, STATE? p. 31-42.

Piet, G.J., C.H.F. Morra, H.A.M. De Kruijf, L.J. Schult'nk and J.G.H. Smeenk. 1981. The behaviour of organic micropollutants during passage through the soil. Stud. Environ. Sci. 17: 557-564.

Popp, J.A. and T.B. Leonard. 1982. The use of in vivo hepatic initiation-promotion systems in understanding the hepatocarcinogenesis of technical grade dinitrotoluene. Toxicol. Pathol. 10(2): 190-196.

Price, C.J., R.W. Tyl, T.A. Marks, L.L. Paschke, T.A. Ledoux and J.R. Reel. 1985. Teratologic evaluation of dinitrotoluene in the Fischer 344 rat. Fund. Appl. Toxicol. 5: 948-961.

Rawlings, G.D. and M. Samfield. 1978. Toxicity of secondary effluents from textile plants. In: Proc. Symp. Process Meas. Environ. Assess. U.S. EPA, Office of Research and Development. EPA-600/7-78-168. NTIS PB290-331.

Rickert, D.E. and R.M. Long. 1980. Tissue distribution of 2,4-dinitrotoluene and its metabolites in male and female Fischer-344 rats. Toxicol. Appl. Pharmacol. 56(2): 286-293.

Rickert, D.E. and R.M. Long. 1981. Metabolism and excretion of 2,4-dinitrotoluene in male and female Fischer 344 rats after different doses. Drug Metab. Dispos. 9(3): 226-232.

Rickert, D.E., R.M. Long and R.W. Tyl. 1980. Urinary excretion and tissue distribution of carbon-14 labeled 2,4-dinitrotoluene in 20-day pregnant Fischer 344 rats. Meeting of the American Society for Pharmacology and Experimental Therapeutics, Rochester, MI. USA, August 17-21, 1980. Pharmacologist. 22(3): 246.

Rickert, D.E., R.M. Long, S. Krakowka and J.G. Dent. 1981. Metabolism and excretion of 2,4-[^{14}C]dinitrotoluene in conventional and axenic Fischer-344 rats. Toxicol. Appl. Pharmacol. 59(3): 574-579.

Rickert, D.E., S.R. Schnell and R.M. Long. 1983. Hepatic macromolecular covalent binding and intestinal disposition of [^{14}C]dinitrotoluenes. J. Toxicol. Environ. Health. 11(4-6): 555-567.

Rickert, D.E., B.E. Butterworth and J.A. Popp. 1984. Dinitrotoluene: Acute toxicity, oncogenicity, genotoxicity and metabolism. CRC Crit. Rev. Toxicol. 13(3): 217-234.

Schut, H.A.J., T.R. Loeb and G.D. Stoner. 1982. Distribution, elimination, and test for carcinogenicity of 2,4-dinitrotoluene in Strain A mice. Toxicol. Appl. Pharmacol. 64(2): 213-220.

Schut, H.A.J., T.R. Loeb, L.A. Grimes and G.D. Stoner. 1983. Distribution, elimination and test for carcinogenicity of 2,6-dinitrotoluene after intraperitoneal and oral administration to Strain A mice. J. Toxicol. Environ. Health. 12(4-6): 659-670.

Schut, H.A.J., R. Dixit, T.R. Loeb and G.D. Stoner. 1985. In vivo and in vitro metabolism of 2,4-dinitrotoluene in Strain A mice. Biochem. Pharmacol. 34(7): 969-976.

Sears, J.K. and N.W. Touchette. 1982. Plasticizers. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 18, 3rd ed., M. Grayson and D. Eckroth, Ed. John Wiley and Sons, Inc., New York. p. 174.

Shils, M.E. and L.J. Goldwater. 1953. Effect of diet on the susceptibility of the rat to poisoning by 2,4-dinitrotoluene. Am. Med. Assoc. Arch. Ind. Hyg. Occup. Med. 8: 262-267. (Cited in Rickert et al., 1984)

Short, R.D., J.C. Dacre and C.C. Lee. 1979. A species comparison of 2,4-dinitrotoluene metabolism in vitro. Experientia. 35(12): 1625-1627.

Simmon, V.F., K. Kauhanen and R.G. Tardiff. 1977. Mutagenic activity of chemicals identified in drinking water. Dev. Toxicol. Environ. Sci. 2: 249-258.

Sina, J.F., C.L. Bean, G.R. Dysart, V.I. Taylor and M.O. Bradley. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat. Res. 113(5): 357-391.

Slaga, T.L., L.L. Triplett, L.H. Smith and H.P. Witschi. 1985. Carcinogenesis of Nitrated Toluenes and Benzenes, Skin and Lung Tumor assays in Mice. Final Report. NTIS DE85012081/WEP. 33 p.

Smith, K.N. 1983. Determination of the reproductive effects in mice of nine selected chemicals: Final Contract Report. NIOSH Contract 210-81-6011, Cincinnati, OH. (Cited in Price et al., 1985)

Smith, J.H. and D.C. Bomberger, Jr. 1981. Volatilization rates of intermediate and low volatility chemicals from water. Chemosphere. 10(3): 281-289.

Soares, E.R. and L.F. Lock. 1980. Lack of an indication of mutagenic effects of dinitrotoluenes and diaminotoluenes in mice. Environ. Mutagen. 2(2): 111-124.

Spanggord, R.J. and B.E. Suta. 1982. Effluent analysis of wastewater generated in the manufacture of 2,4,6-trinitrotoluene: 2. Determination of a representative discharge of ether-extractable components. Environ. Sci. Technol. 16(4): 233-236.

Spanggord, R.J., T. Mill, T.W. Chou, W.R. Mabey, J.H. Smith and S. Lee. 1980a. Environmental fate studies on certain munitions wastewater constituents. Final Report, Phase I - Literature Review. Prepared by Stanford Research Institute, Menlo Park, CA under Contract No. DAMD 17-78-C-8081. U.S. Army Medical Res. and Develop. Command, Fort Derrick, MD.

Spanggord, R.J., T. Mill, T.W. Chou, W.R. Mabey and J.H. Smith. 1980b. Environmental fate studies on certain munition wastewater constituents - laboratory studies. Gov. Rep. Announce. Index (U.S.) 1981. 81(19) 4174. NTIS AD-A099 256.

Spanggord, R.J., K.E. Mortelmans, A.F. Griffin and V.F. Simmon. 1982. Mutagenicity in _Salmonella typhimurium_ and structure-activity relationships of wastewater components emanating from the manufacture of trinitrotoluene. Environ. Mutagen. 4: 163-179.

Spector, W.S., Ed. 1956. Handbook of Toxicology. Vol. I. Acute Toxicities of Solids, Liquids and Gases to Laboratory Animals. W.B. Saunders Co., Philadelphia/London. (Cited in U.S. EPA, 1980a)

SRI (Stanford Research Institute) International. 1985. 1985 Directory of Chemical Producers: United States of America. SRI International, Menlo Park, CA.

Stoner, G.D., E.A. Greisiger, H.A.J. Schut, et al. 1984. A comparison of the lung adenoma response in strain A/J mice after intraperitoneal and oral administration of carcinogens. Toxicol. Appl. Pharmacol. 72(2): 313-323.

Styles, J.A. and M.F. Cross. 1983. Activity of 2,4,6-trinitrotoluene in an in vitro mammalian gene mutation assay. Cancer Lett. 20(1): 103-108.

Tabak, H.H., S.A. Quave, C.I. Mashni and E.F. Barth. 1981. Biodegradability studies with organic priority pollutant compounds. J. Water Pollut. Control Fed. 53(10): 1503-1518.

Tokiwa, H., R. Nakagawa and Y. Ohnishi. 1981. Mutagenic assay of aromatic nitro compounds with Salmonella typhimurium. Mutat. Res. 91(4-5): 321-325.

TSCA-ITC (TSCA Interagency Testing Committee). 1981. Hazard Information Review. Dinitrotoluenes IR-232. Prepared by Environmental Control, The Dynamae Building, Rockville, MD. Prepared under EPA Contract No. 68-01-5789, May 31, 1981.

U.S. EPA. 1977. Computer printout of non-confidential production data from TSCA Inventory. U.S. EPA, Office of Toxic Substances, Washington, DC.

U.S. EPA. 1978. Chemical Hazard Information Profile 2,4-Dinitrotoluene Draft Report. OTS, OPTS, U.S. EPA, Washington, DC. March 9, 1978.

U.S. EPA. 1980a. Ambient Water Quality Criteria for Dinitrotoluene. Office of Water Regulations and Standards. Washington, DC. EPA-440/5-80-045. NTIS PB81-117566.

U.S. EPA. 1980b. Guidelines and Methodology for the Preparation of Health Effect Assessment Chapters of the Ambient Water Quality Criteria Documents. ECAO, OHEA, ORD, U.S. EPA, Cincinnati, OH. [Also published in Federal Register, 45(231): 79347-79357]

U.S. EPA. 1981. Errata for Ambient Water Quality Documents. ECAO, OHEA, ORD, U.S. EPA, Cincinnati, OH.

U.S. EPA. 1983a. Reportable Quantity Document for Benzene, 1-Methyl-2,4-Dinitro-(2,4-Dinitrotoluene). ECAO, ORD, U.S. EPA, Cincinnati, OH. ECAO-CIN-R034.

U.S. EPA. 1983b. Reportable Quantity Document for Benzene, 1-Methyl-2,6-Dinitro-(2,6-Dinitrotoluene). ECAO, ORD, U.S. EPA, Cincinnati, OH. ECAO-CIN-R035.

U.S. EPA. 1984a. MedChem software release 3.32, CLOGP3 computer printout: Graphic Exposure Modeling System (GEMS). U.S. EPA, Office of Toxic Substances, Washington, DC.

U.S. EPA. 1984b. Proposed Guidelines for Carcinogen Risk Assessment. Federal Register. 49(227): 42694-46297.

U.S. EPA. 1985. Computer printout: Fate of atmospheric pollutant (FAP) estimation. U.S. EPA, Office of Toxic Substances, Washington, DC.

USITC (U.S. International Trade Commission). 1983. Synthetic Organic Chemicals. United States Production and Sales, 1982. USITC Publ. 1422, Washington, DC. p. 27.

Vernot, E.H., J.D. MacEwen, C.C. Haun and E.R. Kinkead. 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol. Appl. Pharmacol. 42(2): 417-424. (Cited in U.S. EPA, 1980a)

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., New York. p. 573-574.

Weast, R.C. 1973. CRC Handbook of Chemistry and Physics, 54th ed. CRC Press, Cleveland, OH. p. C-518.

Working, P.K. and B.E. Butterworth. 1984. An assay to detect chemically induced DNA repair in rat spermatocytes. Environ. Mutagen. 6(3): 273-286.

Zepp, R.G., P.F. Schlotzhauer, M.S. Simmons, G.C. Miller, G.L. Baughman and N.L. Wolfe. 1984. Dynamics of pollutant photoreactions in the hydrosphere. Fresentius Z. Anal. Chem. 319: 119-125.

Zoeteman, B.C.J., K. Harmsen, J.B.H.J. Linders, C.F.H. Morra and W. Slooff. 1980. Persistent organic pollutants in river water and groundwater of the Netherlands. Chemosphere. 9(4): 231-249.

APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN Internation
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in August, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values. 4th ed. (Includes Supplemental Documentation, 1981, 1982, 1983). Cincinnati, OH. 486 p.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1985-1986. Cincinnati, OH. 114 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. 2878 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2B. John Wiley and Sons, NY. p. 2879-3816.

Clayton, G.D. and F.E. Clayton, Ed. 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-1983. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., Littleton, MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. WHO, IARC, Lyons, France.

ITII (International Technical Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

NTP (National Toxicology Program). 1984. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

Ouellette, R.P. and J.A. King. 1977. Chemical Week Pesticide Register. McGraw-Hill Book Co., NY.

Sax, N.I. 1979. Dangerous Properties of Industrial Materials, 5th ed. Van Nostrand Reinhold Co., NY.

SRI (Stanford Research Institute). 1984. Directory of Chemical Producers. Menlo Park, CA.

U.S. EPA. 1985. Status Report on Rebuttable Presumption Against Registration (RPAR) or Special Review Process. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

U.S. EPA. 1985. CSB Existing Chemical Assessment Tracking System. Name and CAS Number Ordered Indexes. Office of Toxic Substances, Washington, DC.

USITC (U.S. International Trade Commission). 1983. Synthetic Organic Chemicals. U.S. Production and Sales, 1982, USITC Publ. 1422, Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. Prepared for the U.S. EPA, Washington, DC. PB-269605.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. Office of Pesticide Programs, U.S. EPA, Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.

In addition, the following document was obtained and reviewed:

U.S. EPA. 1978. Chemical Hazard Information Profile on 2,4-Dinitrotoluene, March 9, 1978. OTS U.S. EPA, Washington, DC.